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Although *Succinoides stelliferus*, gen. n., sp. n., *Theba maxima*, sp. n., *Theba longiflagellata*, sp. n. and *Carychium primitivum*, sp. n. are listed as new by Schileyko (MALACOLOGIA 7 (1): 131-132), they were actually described previously; see Schileyko, A. A., 1968, Helicidae (Pulmonata, Stylommatophora) of Talysh [in Russian]. Zoologichesky Zhurnal, 47(3): 337-347.

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MALACOLOGIA

MALACOLOGIA, 1968, 7(1): 1-6

LOSS OF Zn^{65} IN THE MUSSEL *MYTILUS GALLOPROVINCIALIS*[1]

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ABSTRACT

The loss of Zn^{65} from the soft tissues and shells of *Mytilus galloprovincialis* Lam. was investigated in laboratory experiments.

The rate of relative biological loss was higher in the shells than in the soft tissues of *Mytilus* and depended on the time they had been exposed to sea water containing Zn^{65}, being higher after a short contact time. The presence of EDTA[3] (50mg/l) increased the loss of Zn^{65}.

The rate of relative biological loss was not constant but initially more rapid, indicating a multicompartmental zinc metabolism in *Mytilus*.

INTRODUCTION

In an earlier study (Kečkeš, Ozretić & Krajnović, 1968) we have presented the results of some experiments on Zn^{65} uptake in *Mytilus*. It was found that the incorporation of Zn^{65} in these mussels was fast but that equilibrium was reached only after a long period. The uptake rate was found to be about 10 x higher for the soft tissues than for the shells. Furthermore it was demonstrated that the inhibition of Zn^{65} incorporation depended on the EDTA[3] concentration in the experimental basin.

Owing to the importance of Zn^{65} in marine ecology (Rice, 1963) and its role in physiology (Vallee, 1959) these investigations were extended and the loss of the Zn^{65} incorporated in *Mytilus* was studied.

MATERIALS AND METHODS

The *Mytilus galloprovincialis* Lam. used in the experiments were taken from natural habitats and were acclimated to laboratory conditions.

Prior to the loss experiments these mussels were kept for various periods in plastic basins containing aerated sea water to which Zn^{65} was added (1 microCi/l). Carrier free Zn^{65} was prepared by the Institute's cyclotrone and used in the form of chloride (5 milliCi in 5 ml of 10^{-2} N HCl). During the loss experiments the animals were in nonradioactive sea water.

For radiometric determinations a well-type scintillation detector with 2.5 x 2 inch NaI (Tl) crystal (Harshaw) connected to a decade scaler was used.

At different intervals from the beginning of the loss experiments the activities per unit weight of soft tissues and shells were determined separately. The results obtained were corrected for the background radiation, decay of Zn^{65} and sensitivity shift of the instrument.

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[1]This work was supported by contracts with the International Atomic Energy Agency, Vienna (RB/201, RB/201/R1 and RB/201/R2) and the Yugoslav Federal Nuclear Energy Commission, Belgrade (902/27 and 1002/15).

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[3]EDTA = ethylene-diamine-tetraacetic-acid.

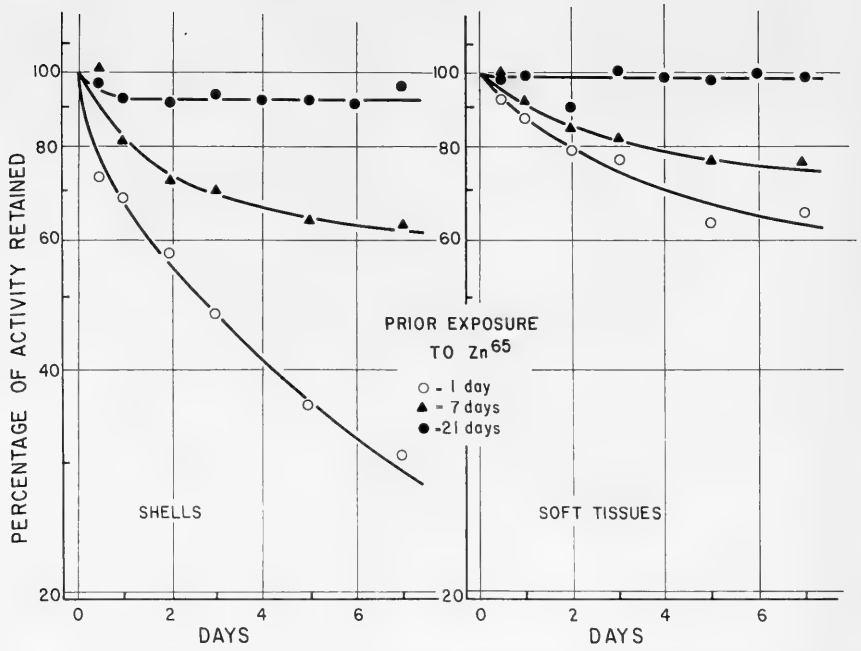

FIG. 1. Relative loss of incorporated Zn^{65} from soft tissues of *Mytilus* after varying periods of uptake. Each mark represents the mean value from at least 4 animals. The curves are fitted by inspection.

EDTA in the form of its disodium salt (Sequestrene NA2)[4] was used as a powerful chelating agent.

RESULTS AND DISCUSSION

If we assume that the incorporated Zn^{65} remains in the animals in a readily exchangeable loosely bound form, we should expect a rapid exchange of Zn^{65} with the stable isotopes of zinc. Soon, however, we realised, that the loss of Zn^{65} is a far slower process than its uptake. The inconsistent results of repeated experiments indicated that the loss depended on the length of exposure of the animals to Zn^{65}. Therefore, so as to obtain a more complete picture of the exchange mechanism and in order to study the effect of prolonging contact with Zn^{65} prior to beginning the loss experiments, several parallel experimental groups were set up.

These experiments showed that the soft tissues of the *Mytilus* bind Zn^{65} more firmly than do the shells and that the time during which the experimental animals were in contact with Zn^{65} had a considerable influence on its loss (Fig. 1).

The loss of Zn^{65} - expressed as percentage of the Zn^{65} content of the animals at the beginning of the loss experiment - was significantly lower both in the soft tissues and in shells when the animals had been in contact with Zn^{65} for a longer period. Thus, for instance, on the 7th day of the loss experiment, only about 3% of the initial activity was lost from the soft tissues of mussels which had previously been in contact with Zn^{65} for 21 days, while about 35%

[4]EDTA was kindly supplied by Geigy Chemical Corporation, USA.

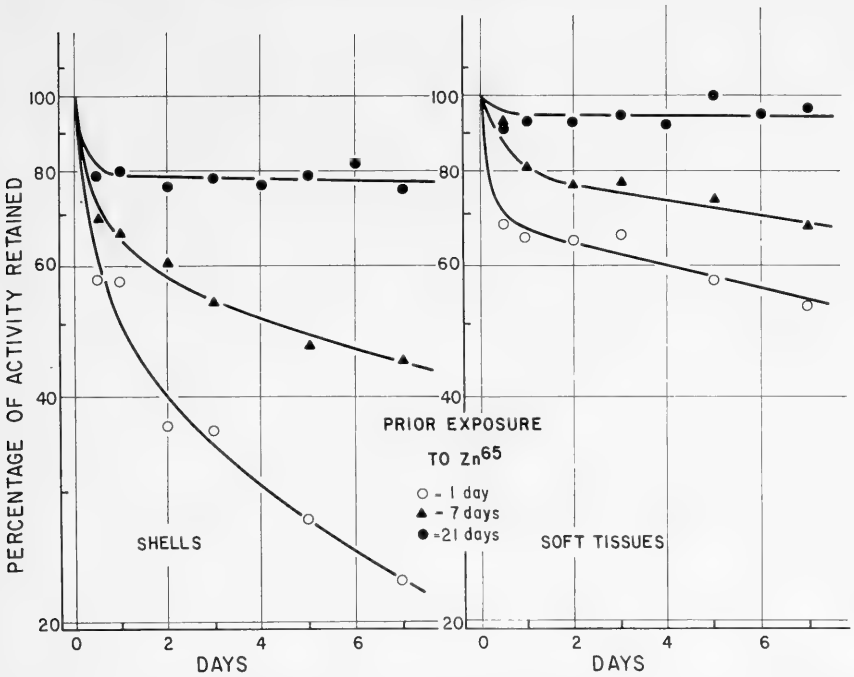

FIG. 2. Relative loss of incorporated Zn^{65} from soft tissues and shells of *Mytilus* in the presence of EDTA (50 mg/l) after varying periods of uptake. Each mark represents the mean value from at least 4 animals. The curves are fitted by inspection.

of the initial radioactivity was lost from those which had had contact with Zn^{65} for only 24 hours. This difference was even more evident when comparing the values obtained for the shells (8% against 70%).

It is interesting to note that the total amount of Zn^{65} lost from the animals in 7 days was approximately the same and did not depend on the duration of exposure to Zn^{65}. However, the fact that the overall loss rate was not constant in a semilogarithmic system indicates more than one mechanism of zinc loss. In general the first parts of the loss curves were much steeper than those following.

In experiments on the loss of Zn^{65} by oysters in the natural environment, Seymour (1966) obtained a relatively constant rate of effective loss (0.55% per day) during 600 days. His results apparently do not concord with ours. However, we did obtain loss curves also very close to the straight line with animals which had been in contact with Zn^{65} for 21 days before the loss experiment.

There are various ways in which Zn^{65} may be bound in and on animal tissues. For example: by adsorption to the free surfaces by organic and inorganic exchange complexes; by incorporation in the crystal lattice of the shells; by inclusion in some organic compounds as a functional component, etc. The initial highest rate of loss in mussels could perhaps be attributed to the removal by exchange of the radiozinc adsorbed on the free surfaces of the animals, while its firm bondage could be explained by a more or less irreversible type of binding, probably within the proteins (in soft tissues) and in the crystal lattice (in shells).

The above loss experiments were complemented by another series of experiments, similar, except that EDTA was added to the sea water used for

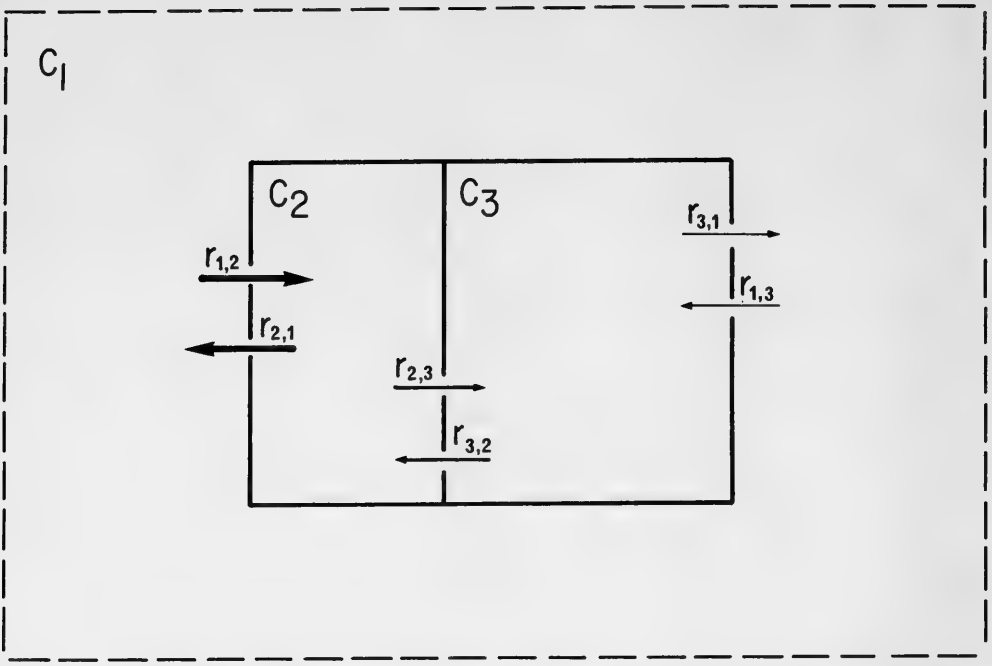

FIG. 3. A simplified scheme of zinc transport in *Mytilus*. C_1, C_2, C_3, hypothetical "compartments", C_1 being the environmental sea water and C_2, C_3 the animal; r, rates for zinc transfer between compartments ($r_{1,2}$ meaning the transfer rate from C_1 to C_2). Further explanations in the text.

"decontamination" in the proportion of 50 mg/l. The results of these experiments (Fig. 2) showed that EDTA did indeed affect the zinc loss, hastening it significantly. The differences between the groups exposed to Zn^{65} for various periods, already observed in the loss experiments without EDTA, were even more clearly evident. Again the loss of Zn^{65} was relatively higher in animals that had been in contact with the radio-isotope for only a short period.

On the basis of the uptake and loss experiments the kinetics of the zinc metabolism in mussels can be schematically simplified as an exchange in a multicompartmental system with various transfer rates between the compartments (Fig. 3).

Let us assume the sea water outside the animal to represent 1 "compartment" (C_1) and postulate the presence of 2 hypothetical "compartments" (C_2 and C_3) within the soft tissues and shell of the animal, each with a certain quantity of zinc (M_1, M_2 and M_3), Zn^{65} (R_1, R_2 and R_3) and with various transfer rates of zinc (r) between them. During the uptake and loss experiments the size of the 3 compartments (C_1, C_2 and C_3), their zinc content (M_1, M_2 and M_3), the transfer rates of zinc between the compartments (r) and the Zn^{65} content of C_1 (R_1) do not change appreciably. Compartment C_2 holds the adsorbed zinc and serves as the main source of zinc for compartment C_3 which binds zinc in a more or less irreversible way. The zinc (and Zn^{65}) transfer rates between

C_1 and C_2 ($r_{1,2}$ and $r_{2,1}$) are nearly equal, but $r_{1,2}$ is greater than the transfer rate of zinc from C_2 to C_3 ($r_{2,3}$), while $r_{2,3}$ is equal to or greater than the transfer rate of zinc from C_3 to C_2 ($r_{3,2}$). The exchange between C_1 and C_3 ($r_{1,3}$ and $r_{3,1}$) is very small or not existent.

According to this scheme R_2 and R_3 increase during the uptake experiment, and thereby the ratios R_2/M_2 and R_3/M_3, until at equilibrium they are equal to R_1/M_1. Since the exchange between C_1 and C_2 is faster, the situation $R_2/M_2 = R_1/M_1$ will be obtained prior to $R_3/M_3 = R_1/M_1$.

In loss experiments the ratio R_2/M_2 will tend to equilibrate with R_1/M_1 faster than the ratio R_3/M_3 because the loss of R_3 from C_3 will be slower than the loss of R_2 from C_2.

The differences in the loss curves obtained from animals exposed to Zn^{65} for various periods could be explained by the differences between the specific isotopic content in C_2 and C_3 (i.e. R_2/M_2 and R_3/M_3) at the beginning of the experiment. It is obvious that the overall loss can proceed in a comparable manner only when R_2/R_3 is initially the same.

EDTA can only sequestrate the adsorbed Zn^{65} directly from the animal (R_2 from C_2) or indirectly by removing the available zinc from sea water (M_1 from C_1), and therefore its effect is greater when the uptake has lasted only a short period because it takes time for R_2 to become R_3.

The given scheme can be taken as a rough speculation only, because one should expect that the actual number of zinc "compartments" in *Mytilus*, and therefore the number of possible interactions between them, is higher than here assumed. Unfortunately, our experimental data, partly due to the error inherent in the method, are insufficient to calculate the Zn^{65} exchange rates and estimate the number of main compartments, but the system described can be used as a basic idea in further work.

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The authors wish to express their gratitude to Miss Maja Benetta, Miss Marija Marečić and Mr. Slavko Dragić for their valuable technical assistance.

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RÉSUMÉ

ELIMINATION DE ZN^{65} CHEZ LA MOULE *MYTILUS GALLOPROVINCIALIS* LAM.

S. Kečkeš, B. Ozretić et M. Krajnović

L'élimination de Zn^{65} à partir des parties molles et de la coquille de *Mytilus galloprovincialis* est étudiée expérimentalement au laboratoire.

Proportionnellement l'élimination biologique est plus élevée dans la coquille que dans les parties molles et dépend du temps d'exposition des *Mytilus* dans l'eau de mer contenant Zn^{65}. Pour un temps court l'élimination est plus forte. La présence de l'E.D.T.A. (50 mg/1) augmente l'élimination de Zn^{65}.

La valeur relative de l'élimination biologique n'est pas constante, mais plus rapide initialement, ce qui indique un métabolisme du zinc multiphasique chez *Mytilus*.

RESUMEN

PERDIDA DE ZN^{65} EN EL MEJILLON *MYTILUS GALLOPROVINCIALIS*

S. Kečkeš, B. Ozretić y M. Krajnović

En experimentos de laboratorio la pérdida biológica de ZN^{65} fué más elevada en las conchas de los mejillones que en los tejidos blandos, y dependió del tiempto en que habian sido expuestas a agua de mar conteniendo ZN^{65} siendo mayor después de un corto contacto. La presencia de EDTA (50 mg/1) aumentó la pérdida de ZN^{65}. Las pérdidas no fueron constantes, sino más rápidas al principio, indicando un metabolismo de zinc multicompartamental en *Mytilus*.

EDTA = ácido etileno-diamino-tetracético

АБСТРАКТ

ПОТЕРЯ Zn^{65} У МИДИЙ *MYTILUS GALLOPROVINCIALIS*

С. КИКС, Б. ОЗРЕТИЋ и М. КРАЙНОВИЧ

Путём лабораторных экспериментов исследовалась потеря Zn^{65} из тканей тела и из раковины *Mytilus galloprovincialis*.

Относительная скорость потери Zn^{65} биологическим путем была больше в раковине мидии, чем в тканях её тела и зависела от продолжительности её содержания в морской воде с Zn^{65}. При более кратковременном содержании моллюска в такой воде потери были более высокими. присутствие EDTA (этилен-диамин-тетрауксусной кислоты) в количестве 50 мг/л, увеличивало потерю Zn^{65}.

Скорость относительной потери Zn^{65} биологическим путём была не постоянна, а начальная её скорость была, обычно, более высокой, что указывает на многосторонность метаболизма цинка у мидий.

MALACOLOGIA, 1968, 7(1): 7-15

UNISEXUALITY IN THE PARASITIC FAMILY ENTOCONCHIDAE (GASTROPODA: PROSOBRANCHIA)

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ABSTRACT

A thorough examination of the formation of the so-called testis in *Enteroxenos oestergreni* Bonnevie, a gastropod endoparasite of the sea-cucumber *Stichopus tremulus,* has definitely shown that it is not a true male gonad. It arises by the implantation of a pygmy male in the wall of the parasite's pseudopallial cavity. As a small, ciliated larva that has lost its shell, the male enters the female through the ciliated tubule, which, in young females, connects their pseudopallial cavity with the lumen of the host's oesophagus. It attaches to a special pseudopallial organ, or male receptacle, formerly regarded as the first rudiment of the testis. Having lost its ciliation, the male penetrates the receptacle and expands into the "testis". It is argued that the so-called testis in the closely allied genus *Thyonicola* also represents a pygmy male, and that the poorly known genus *Entoconcha* will likewise prove to be dioecious, as *Entocolax,* the 4th member of this group, is known to be. The significance of these considerations on the classification of these endoparasites is evident: a grouping into 2 separate families, Entoconchidae and Enteroxenidae, which was largely based on the supposed hermaphroditic nature of the latter, no longer seems justified, nor is an assignment to the opisthobranchs, as proposed by certain authors.

INTRODUCTION

The gastropod family Entoconchidae is generally considered to include *Entoconcha* Müller 1852 (= *Heliocosyrinx* Baur 1864) and *Entocolax* Voigt 1888, internal parasites of synaptid holothurians, *Enteroxenos* Bonnevie 1902 (= *Comenteroxenos* Tikasingh 1961, according to Kincaid, 1964) a parasite of aspidochirote holothurians, and *Thyonicola* Mandahl-Barth 1941 (= *Parenteroxenos* Ivanov 1945), which is parasitic upon dendrochirote holothurians. Schwanwitsch (1917) proposed a subgrouping into Entoconchini (*Entoconcha* and *Entocolax*) and Enteroxenini, and Mandahl-Barth (in Heding & Mandahl-Barth, 1938) even denied that a closer affinity existed between *Entoconcha* and *Entocolax* on the one hand, and *Enteroxenos* on the other, and established a new family, Enteroxenidae, for the latter, to which he later added *Thyonicola.* Tikasingh & Pratt (1961) also recognize 2 families: Entoconchidae comprising *Entoconcha* and *Entocolax*, and Enteroxenidae comprising *Enteroxenos*, *Comenteroxenos* and *Thyonicola.* In this paper, for reasons that will appear later, the group will be treated as a single taxonomic unit, i.e. as the family Entoconchidae.

The general structure of these remarkable parasites (Fig. 1) is fairly well-known, mainly from the work of Baur (1864), Voigt (1888), Bonnevie (1902), and Ivanov (1947, 1948, 1949a). It here will suffice to mention that all species are generally oblong or vermiform and have no shell, except in the larval state. A rudimentary intestine is present in *Entoconcha* and

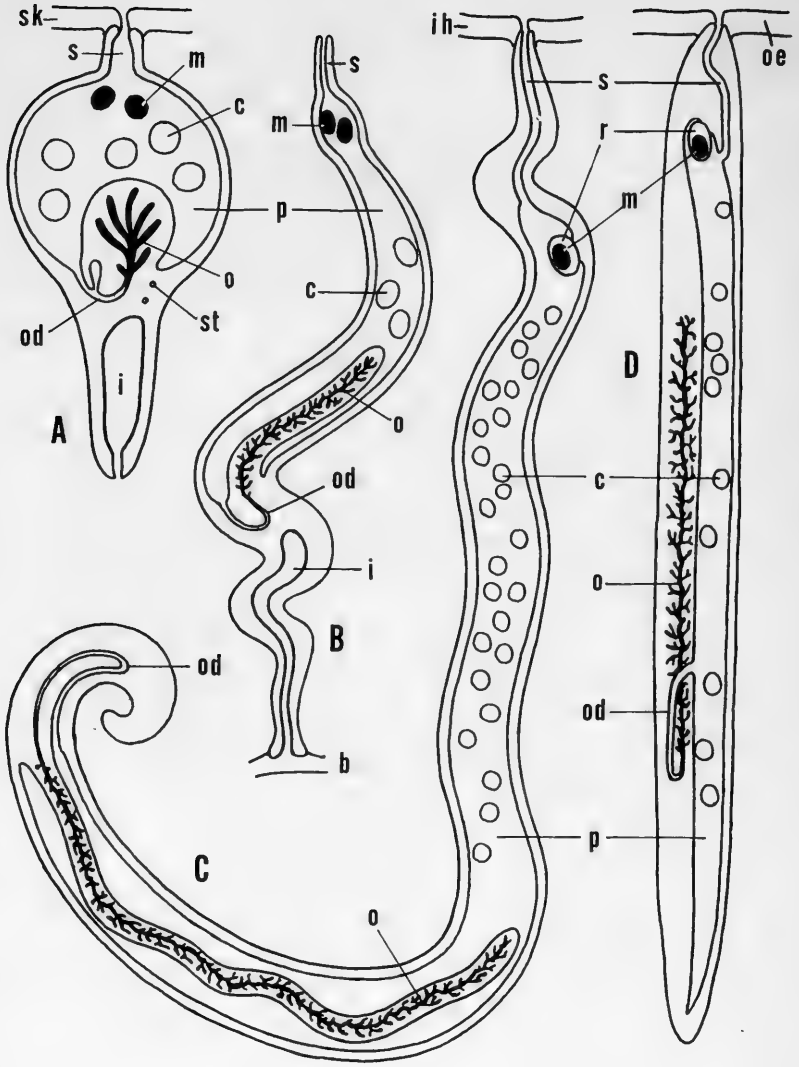

FIG. 1. Comparative anatomy of the family Entoconchidae. A, *Entocolax;* B, *Entoconcha;* C, *Thyonicola;* D, *Enteroxenos;* redrawn, and slightly modified, from Ivanov (1945). A and B: x 3, C and D: x 2.

b blood vessel of host's intestine
c egg-capsules
i intestine
ih intestine of host
m dwarf male
o ovary
od oviduct
oe oesophagus of host
p pseudopallial cavity
r male receptacle
s siphon in A and B, resp. ciliated tubule in C and D
sk skin of host
st statolith

Entocolax; a central cavity, or pseudopallial cavity occurs in all forms; a visceral sac containing the ovary, may protrude into it. All species practice brood protection, the eggs being deposited in capsules that accumulate in the pseudopallial cavity. This cavity communicates with the exterior by way of a short canal, or siphon, in *Entoconcha* and *Entocolax*, and, in *Enteroxenos* and *Thyonicola*, through a long and delicate ciliated tubule, that has been observed to degenerate in older parasites, at least in the former genus.

Whereas *Entocolax* is clearly dioecious with a pronounced sexual dimorphism, the dwarf males living in the pseudopallial cavity of the females (m, Fig. 1 A), *Entoconcha* was considered a hermaphrodite by its discoverer, Müller (1852), and also by Baur (1864), a view that was supported by Bonnevie (1902). The hermaphrodite nature of the latter was, however, questioned by Schiemenz (1889) and by Schwanwitsch (1917) in the light of the unquestionable find of pygmy males in *Entocolax schwanwitschi* (Schwanwitsch, 1917). The 2 authors pointed out that the sperm-containing spherules present in the pseudopallial cavity of *Entoconcha* might just as well represent dwarf males (m, Fig. 1 B) as a testis. This point as well as the general microanatomy of the single species, *E. mirabilis*, needs reinvestigation. There has been general agreement among all investigators that *Enteroxenos* as well as *Thyonicola* are hermaphrodites. Regarding *Entocolax* Tikasingh & Pratt (1961) express a rather unorthodox view - based upon reasons not obvious to the present author - i.e. that the genus might be a protandrous hermaphrodite.

Such conflicting opinions regarding the sexuality of the various genera have arisen mainly from the fact that they are founded, with few exceptions, on assumptions rather than on thorough examinations. It was therefore planned to inspect the structure of the so-called testis more closely, and, in particular, to reinvestigate its mode of formation. *Enteroxenos oestergreni* Bonnevie was chosen as the first object of this investigation, because it was the most easily available to the author. The animals were collected at Drøbak in the Oslo Fjord, Norway, where they commonly occur within the large aspidochirote, *Stichopus tremulus* (Gunnerus), at depths of between 65 and 200 meters. The parasites are usually attached to the oesophagus of the host, and occasionally to the cloacal wall, respiratory tree, gonads or large intestine, and eventually detach themselves to float freely in the body cavity. Collections were made at all times of the year, and stages of all sizes have been secured. The material was fixed in Bouin's fluid and stored in 70% alcohol.

OBSERVATIONS

The structure of the testis in mature specimens of *Enteroxenos oestergreni* has been described very carefully by Bonnevie (1902, 1906) and does not differ essentially from that found in other species of *Enteroxenos* (Tikasingh, 1962) or *Thyonicola* as described by Mandahl-Barth (1941), Ivanov (1947) and Tikasingh (1961). In both genera it forms an irregular projection from the wall of the central pseudopallial cavity (p) very close to where the ciliated tubule (s) opens into it (Fig. 1, C & D). In *Enteroxenos oestergreni* the projection is said to contain a single vesicle which is richly subdivided. In *E. parastichopoli* (Tikasingh), *Thyonicola mortenseni* Mandahl-Barth, and *T. dogieli* (Ivanov) a single vesicle is similarly present, whereas the number of vesicles in *T. americana* Tikasingh is said to vary up to a maximum of 5. In *Entoconcha mirabilis* Müller several vesicles normally occur (m, Fig. 1, B), but the question of whether they are attached to the pseudopallial wall, or independent, has never been settled satisfactorily. According to Bonnevie the wall separating the testis from the pseudopallial

cavity in *Enteroxenos* is very thin and formed by 3 epithelia, that are most clearly distinguishable in the young and still immature testis. Externally there is a cubical non-ciliated epithelium, continuous with that lining the pseudopallial cavity. Under this epithelium lies a distinct basal membrane which completely surrounds the testicular vesicle. Next follows a syncytial epithelium with a few and scattered oval or flattened nuclei; on its inner side is a layer of scattered cells with comparatively large and spherical nuclei. These are the germ-cells, which give rise to the spermatogonia through division by mitosis. As could be expected from Bonnevie's painstaking description, the account is indisputably correct, and I can add nothing from the examination of my own slides. Attention was therefore focused on the first stages in the development of the testis.

Bonnevie has shown that the epithelium of the pseudopallial cavity, whose function it is to surround the testicular vesicle, begins specializing at a body length of no more than 1.5 mm. The epithelial cells elongate and fold into a series of close-set projections, supported by a central core of mesenchymatous tissue. This process continues, still according to Bonnevie, until the body length approaches approximately 40 mm. On this point also I can establish that her description is very precise.

The crux of the matter is, however, the transformation of this so-called rudimentary testis into one in which the above-mentioned stratification of layers is demonstrable. According to Bonnevie (1902) this change takes place at a body length of 40-50 mm, though it may occasionally occur earlier: in her later paper (1906) a section is shown through a parasite of 27 mm, in which the transformation has already taken place. Quoting Bonnevie, the transformation is very sudden (1902: 758): "Diese Veränderung des Aussehens der Hodenanlage geht sehr schnell vor sich; es ist daher mit der grössten Schwierigkeit verbunden, sich eine vollständige Serie der verschiedenen Stufen dieser plötzlichen Umwandlung zu verschaffen."* Nevertheless, she claims to have observed exactly how it comes about: by the fusing of the basal membranes of the epithelium in the proximal part of the rudiment, the central core of mesenchymatous tissue is separated off; however, before the fusion is completed, a mesodermal syncytium, that has arisen from undifferentiated mesenchymatous cells, moves into the vesicle so formed and spreads over its walls, lying everywhere under the epithelial basal membrane. The syncytium thereafter differentiates into 2 layers, an outer one with a few oval nuclei, and an inner one, the germ cell layer, with rather numerous, spherical nuclei. According to Ivanov (1949a: 123, Figs. 16-21) the development of the testis in *Thyonicola* takes a similar course, since it is claimed to arise from a mesodermal rudiment which penetrates into the epithelial folds and is later partitioned off from the connective tissue of the body.

To check the accounts given on this point, a great number of parasites from 7 - 100 mm long were examined. The proximal part of the body containing the organ in question was removed and sectioned at 6, 8 or 10μ. A study of these sections, combined with microdissections of other parasites, definitely showed quite another origin of the male germ-cells than was hitherto supposed.

First of all it soon became apparent that a typical vesicle in which all 3 layers were distinguishable can appear at a much earlier stage than reported by Bonnevie (the smallest parasite showing such a vesicle measured 10 x 1 mm). Next, it appeared that quite frequently it did not seem to develop at all. Most important, however, was the finding

*"This change in the aspect of the testicular rudiment proceeds exceedingly fast; it is therefore most difficult to obtain a complete series of the different stages of this sudden transformation." (Translation)

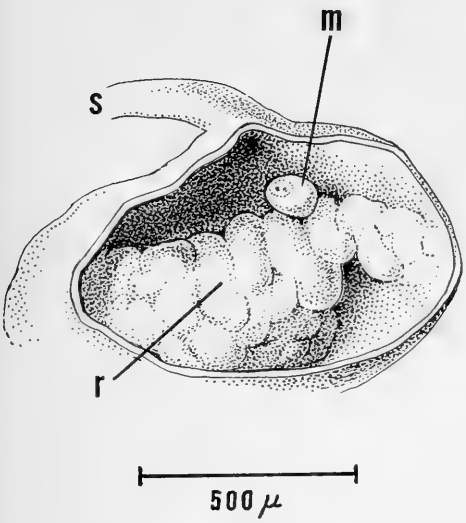

FIG. 2. Proximal part of the pseudopallial cavity of an immature female of *Enteroxenos oestergreni* (22 x 1. 5 mm) cut open. (m) larva which has penetrated the ciliated tubule (s) and settled upon the male receptacle (r).

confirming the author's suspicions, that the germ cells did not arise in the parasite itself. They are supplied from outside in the form of tiny larvae, which, after they have entered through the ciliated tubule, settle upon the "testicular" rudiment (r, Fig. 1, C, D).

Proof of this assertion is furnished by the identification of minute ciliated larvae in the central cavity of 7 young parasites (lengths between 10 and 37 mm). These larvae had no shells and were almost similar to the postmetamorphosed larva of *Thyonicola*, described by Ivanov (1948: Fig. 1, C) except that the operculum had been lost. One single larva was recorded in each instance. The larvae were found in the immediate vicinity of the "testicular" rudiment, or, in a few cases, in direct contact with its surface (Fig. 2). In 2 other cases the larvae had lost the ciliation and were firmly attached to the rudiment, obviously engaged in penetrating into it, or rather perhaps being enclosed by it. In other instances, however, these larval organisms had been transformed into small spheres with a volume only slightly exceeding that of the larvae, but structurally similar to the young "testis", and were superficially placed in the "testicular rudiment". Further proof of the identity of these spheres with the larvae is given by the invariable presence of 2 statocysts, which, however, disappear later on.

These observations can be interpreted in only one way: the sexes are separate in *Enteroxenos*. The parasite, hitherto supposed to be a hermaphrodite, is actually a female and the fertilization of its eggs is accomplished by a dwarf male. The male enters the ciliated tubule as a larva and attaches itself to a small area of the inner wall of the pseudopallium specialized as a male receptacle. It subsequently penetrates the receptacle to become incorporated in the female body as an integral part, whereupon, probably while being nourished by the female, it expands into the "testis". These observations have thus invalidated the findings of Bonnevie both as to the nature of the "testis" and as to how it arises. A later contribution will be devoted to an exhaustive account of the metamorphosed larva and its transformation into the full-grown male.

DISCUSSION

The observations on the sexuality of *Enteroxenos* set forth in the present paper involve a number of new insights, some of which will be summarized briefly in the following.

We now at last have a definite answer as to the function of the ciliated tubule, which in *Thyonicola* and *Enteroxenos* connects the pseudopallial cavity and the host's oesophagus. Since the latter is filled with sea water and mud, it is probably not, as advanced by Bonnevie, a nutritional canal in the young, growing parasite, nor is it a remnant of a former gonoduct (Tikasingh, 1962). Whether or

not it has an accessory function, such as, for instance, to serve as an exit for the larval capsules in *Thyonicola*, as was suggested by Ivanov (1948), its main role is evidently to make possible the entry of the potential dwarf males into the pseudopallial cavity of the young female. Such a function also explains in a most satisfactory way why the sealing off and degeneration of the tubule, which Bonnevie (1902) observed in *Enteroxenos oestergreni*, and Tikasingh (1962) in *E. parastichopoli*, actually never starts until after the parasite has reached sexual maturity.

It would be expected that some female parasites never receive a male in the course of their life. One would a priori suppose that parasites, which for one or another reason happen to be attached to their host in an abnormal position (i.e. the respiratory tree, the cloacal wall, etc.) will have a much smaller chance of being visited by potential males than those fixed to the oesophagus, which is the usual habitat of the species. Preliminary observations have confirmed that this reasoning is correct: the great majority of the parasites so far examined that were fastened to the cloaca had indeed never received a male. There are also indications that this situation leads to a degeneration of the male receptacle, a speedier detachment of the parasite and its ultimate death.

The demonstration of *Enteroxenos oestergreni* as a unisexual, not hermaphroditic, animal, in which the males are pygmies, brings it on a par with *Entocolax*. The indisputable relationship between *Thyonicola* and *Enteroxenos* and, in particular, the identical structure of the organ hitherto considered to be the testis, makes it self-evident that *Thyonicola* is likewise unisexual, even if direct observations are lacking. Further, if we accept the combined theories of Schiemenz (1889) and Bonnevie (1902) i.e. that *Entocolax*, *Entoconcha* and *Enteroxenos* are related and form an evolutionary series in which *Entoconcha* represents an intermediate stage, it is a foregone conclusion that the so-called testis in that genus which has not been thoroughly investigated will prove to be nothing else but a pygmy male, even though the presence of a receptacle for these males remains yet to be demonstrated.

Finally, the effect of the new understanding of sexuality in these endoparasites upon the systematics within the group, as well as on the position of the group among the other gastropods, needs to be discussed. The criterion for the separation of the Entoconchidae from the Enteroxenidae which, according to Mandahl-Barth (1941) is the most basic, resides in their dioecious, respectively hermaphroditic nature. This character of distinction has now been invalidated by demonstrating the unisexuality in *Enteroxenos* and deducing it, by analogy, in the similar *Thyonicola*, while the fact that dwarf males have now been shown to occur in the latter family also has provided a further character in common. Since Ivanov (1949b) has rightly minimized the significance of the difference claimed to exist in sperm morphology, the only important differences between these families left are that, (1) the larval shell in *Entocolax* and *Entoconcha* has a tendency to be spiral, which it has not in *Enteroxenos* and *Thyonicola*, and that (2) an intestine is present in the former 2 genera, whereas it has been lost in the latter 2. In view of the above considerations the author very much doubts the advisability of maintaining 2 separate families.

The opinions advanced by Tikasingh & Pratt (1961) with regard to the systematic position of these endoparasites within the class Gastropoda deserve comment, since they have been unquestioningly adopted by various authors, including Taylor & Sohl (1962) in their systematic review and Hyman (1967) in her treatment of the invertebrates. Tikasingh & Pratt claim that the Entoconchidae and Enteroxenidae are opisthobranchs, basing themselves on Fischer (1883). True, Fischer did refer the entoconchids to the Opisthobranchia, but did so at a time when only the very super-

ficially known *Entoconcha* had been described. These authors further quote Mandahl-Barth (1941) as having also done so, disregarding the fact that he was discussing only *Thyonicola* and *Enteroxenos*. Mandahl-Barth lists as opisthobranch characteristics: the shape of the larval shell, the presence of very yolky eggs, and, above all, hermaphroditism. However, the observations presented in the present paper have robbed the argument of its main basis, while the 2 remaining characters do not seem specific enough for classificatory purposes. But even if we accepted Mandahl-Barth's argumentation regarding the 2 genera formerly regarded as hermaphroditic, the evidently dioecious *Entoconcha* and *Entocolax* still could not be considered opisthobranchs, a conclusion also reached by Mandahl-Barth. Possibly in an attempt to partially overcome this difficulty, Tikasingh & Pratt have equipped these latter 2 genera with an opisthobranch character by suggesting that the dwarf males found in their pseudopallial cavity are not true males, but "have arisen from the gonad of the parasitic form, as is common among protandric hermaphrodites". There is no evidence for such a statement which rests upon speculation.

Tikasingh & Pratt further state that the systematic position of these endoparasitic gastropods is difficult to determine, because almost all organ systems are absent in the adult stage. However, since Schiemenz (1889), Vaney (1913), and other authors have brought together a bulk of evidence in favour of the view that these endoparasites form the climax of an evolutionary series which has started within the family Eulimidae (Melanellidae), the question is considerably simplified. If their views are accepted one needs only determine whether the Eulimidae should be placed among the Prosobranchia or the Opisthobranchia, which is fairly easy, because the eulimids have been only slightly modified as a result of their parasitic way of life. Tikasingh & Pratt have not taken up any position in respect to this point, but as far as the writer is aware, no objections have ever been raised to the view that the Eulimidae are true prosobranchs. So are the Styliferidae which according to Schiemenz' theory link the Eulimidae with the Entoconchidae. Thus it is not surprising that by far the majority of students of the Entoconchidae have referred this family to the Prosobranchia.

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RÉSUMÉ

GONOCHORISME DANS LA FAMILLE PARASITE DES ENTOCONCHIDAE (GASTROPODA: PROSOBRANCHIA)

J. Lützen

Un examen approfondi de la formation du soi-disant testicule chez *Enteroxenos oestergreni* Bonnevie, un gastropode endoparasite d'une holothurie *Stichopus tremulus*, a montré de façon définitive qu'il ne s'agit pas d'une vraie gonade mâle. Cela commence par l'implantation d'un mâle nain dans la paroi de la cavité pseudopalliale du parasite. Sous la forme d'une petite larve ciliée qui a perdu sa coquille, le mâle pénètre dans la femelle par le tubule cilié, qui, chez les jeunes femelles, met en connexion la cavité de leur pseudopallium avec la lumière de l'oesophage de l'hôte. Il s'attache à un organe spécial du pseudopallium, ou réceptacle du mâle, jusqu'ici considéré comme le premier rudiment du testicule. Après avoir perdu sa ciliature, le mâle pénètre dans le réceptacle et s'étend dans le "testicule." Il est démontré que le soi-disant testicule du genre très voisin *Thyonicola* correspond aussi à un mâle nain, et que le genre mal connu *Entoconcha* se révèlera de la même façon gonochorique, tout comme *Entocolax*, le quatrième membre de ce groupe. La signification de ces observations pour la classification de ces endoparasites est évidente: un classement en deux familles séparées Entoconchidae et Enteroxenidae, qui était en grande partie basé sur la nature hermaphrodite supposée de la dernière, ne semble désormais plus justifié, pas plus qu'un rapprochement avec les Opisthobranches, comme certains auteurs le proposaient.

RESUMEN

UNISEXUALIDAD EN LA FAMILIA PARASITA ENTOCONCHIDAE (GASTROPODA: PROSOBRANCHIA)

Jorgen Lützen

Un examen completo de la formación del llamado testis en *Enteroxenos oestergreni* Bonnevie, gastrópodo endoparásito de la holoturia *Stichopus tremulus*, comprobó definitavemente que no constituye una verdadera gonada masculina, sino que es producido por la implantación de un macho pigmeo en la pared de la cavidad pseudopaleal del parásito. Como una pequeña larva ciliada que ha perdido su conchilla, este diminuto macho entra por el tubito ciliado que en las hembras jóvenes conecta la cavidad pseudopaleal con el lúmen (conducto) esofágico del huésped. Se fija a un órgano pseudopaleal especial, o receptaculo masculino, antiguamente considerado como el primer rudimento del testículo. Habiendo perdido las cilias, el macho penetra el receptaculo y se expande dentro del "testis." Se discute que el también llamado testis en el género muy afin *Thyonicola* representa igualmente un macho pigmeo, y que el menos conocido género *Entoconcha* de la misma manera probaría que es dioico, como *Entocolax*, el 4º miembro de este grupo, se sabe que es. El significado de estas consideracions sobre la clasificación de tales endoparásitos es evidente: no puede justificarse ya la separación de 2 familias Entoconchidae y Enteroxenidae, que se basaban principalmente sobre la naturaleza hermafroditica del último, ni tampoco se pueden asignar a los opistobranquios como fuera propuesto por ciertos autores.

АБСТРАКТ

ОБОДНОПОЛОСТИ У ФОРМ ИЗ ПАРАЗИТИЧЕСКОГО СЕМЕЙСТВА ENTOCONCHIDAE (GASTROPODA: PROSOBRANCHIA)

Г. ЛЮТЦЕН

Исследование образования так называемого семенника (testis) у *Enteroxenos oestergreni* - эндопаразитческой гастроподы, обитающей в *Stichopus tremulus*, ясно показало, что это не настоящая мужская гонада. Она возникает благодаря внедрению карликового самца в стенку псевдопаллиальнойполости самого паразита. Ввиде маленькой ресничатой личинки, утратившей свою раковину, самец проникает в самку через ресничатую трубочку, которая у молодых самок связывает их псевдопаллиальную полость с просветом пищевода хозяина. Он прикрепляется к специальному псевдопаллиальному органу или мужскому рецептакулу, ранее считавшемуся первым рудиментом семенника. Потеряв свои реснички, самец проникает в рецептакул и превращается в "семенник" ("testis"). Было доказано, что так называемый тестис у очень близкого рода *Thyonicola* также представляет собой карликового самца и что мало изученный род *Entoconcha* будет, видимо, известен как раздельнополый, как и *Entocolax*, четвёртый член этой группы моллюсков. Значение этих фактов для классификации указанных моллюсков - эндопаразитов очевидно: разделение на 2 отдельных семейства - Entoconchidae и Enteroxenidae, которое было основано в большой степени на предполагаемом гермафродитизме последних, больше, видимо, не подтверждается, как и их отнесение к Opisthobranchia, как это предлагалось различными авторами.

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A SYSTEMATIC STUDY OF *ONCOMELANIA HUPENSIS CHIUI* (GASTROPODA: HYDROBIIDAE)[1]

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ABSTRACT

The hydrobiid snail which Chiu (1961) reported to be the first intermediate host of *Paragonimus iloktsuenensis* in Taiwan (Formosa) was described as *Tricula chiui* by Habe & Miyazaki (1962). The snail, found in an isolated basin in northern Taiwan (A-li-lao area), was later shown to be susceptible to *Schistosoma japonicum* also (Chiu, 1965b, 1967). Anatomical data have now shown this snail to belong to the genus *Oncomelania*. It is closely allied to the subspecies of *O. hupensis,* as described for *O. h. formosana* (Davis, 1967) and for *O. h. nosophora* and *O. h. quadrasi* (Davis, unpublished), because: 1) the verge has a papilla and characteristic strip of cilia near the tip; 2) the seminal vesicle is knotted; 3) the spermathecal and sperm ducts arise from the right lateral edge of the bursa copulatrix near the anterior end as 2 separate tubes bound together in a common connective tissue sheath; 4) the oviduct coils over the seminal receptacle in a distinct manner; 5) the structure of the male and female gonad is similar; 6) the shell is distinctly of the *Oncomelania* type in spite of the obsolete varix.

Immunological studies using micro-Ouchterlony double diffusion tests with absorbed antiserum (anti- *O. h. formosana* foot muscle extract) indicated that *"Tricula chiui"* was more closely associated with populations of *O. h. formosana* than with other subspecies of *Oncomelania hupensis* because: 1) all antigen-antibody systems were homologous between them; 2) they had systems not present in or only partially identical with those occurring in the other subspecies of *O. hupensis*.

Polyacrylamide electrophoresis of proteins from foot muscle extract of *"T. chiui"* and several populations of *O. h. formosana* showed that the former had a densitometric profile of the separated protein components more similar to 1 population of *O. h. formosana* from N. E. Taiwan (I-lan county) than to any other.

Hybridization studies showed that *"T. chiui"* produced fertile hybrids with *O. h. formosana*.

From these collected data it appears most likely that the snail originally named *"Tricula chiui"* had its origin from stock giving rise to current *O. h. formosana* from I-lan county and that with subsequent isolation near the edge of the sea at A-li-lao, where tall mountains separate them from the I-lan region,

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this stock evolved characteristics justifying subspecific status, namely, 1) a much shortened shell, 2) significantly fewer gill filaments than other subspecies of *O. hupensis*, 3) shell with obsolete varix, 4) a longer pleuro-supraesophageal connective than found in *O. h. formosana*, 5) a larger supraesophageal ganglion, 6) a difference in frequency of cusp number on the various teeth of the radula. Accordingly the taxon is named *Oncomelania hupensis chiui*.

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INTRODUCTION

Chiu (1961) reported on the snail host of the lung fluke, *Paragonimus iloktsuenensis*, in Taiwan (Formosa). This undescribed snail, found by Chiu on the 24th of April, 1961 at A-li-lao village (Fig. 1), was subsequently described by Habe & Miyazaki (1962) as *Tricula chiui*. Further reports, mainly on the parasite, were made by Chiu (1962) and Miyazaki & Chiu (1962). Reports on the parasitological aspects involving the molluscan intermediate host were presented by Chiu (1965a, b, 1967), who showed that this snail was also capable of transmitting *Schistosoma japonicum*. The usual intermediate host of this blood Fluke is *Oncomelania*, a hydrobiid genus of the subfamily Pomatiopsinae.

Habe & Miyazaki (1962) stated that *Tricula chiui* differed from other species of *Tricula*, a genus in the Hydrobiidae, subfamily Triculinae, in having a broadly oval shell and that it was an ally to *"Katayama (=Oncomelania) formosana."*

In June 1963, Dr. Chiu found in the collection at the Mollusk Division, University of Michigan, an alcoholic lot of specimens collected by Dr. Robert E. Kuntz from A-li-lao, Taiwan. The lot was without specific designation and was recognized as *Tricula chiui*. I examined the anatomy and found a striking resemblance to the anatomy of the so-called species of *Oncomelania* (*O. formosana*, *O. nosophora*, *O. quadrasi* and *O. hupensis*) presently considered to be subspecies of *O. hupensis* (Davis, 1967). At that time studies were initiated to assess the relationship of *"Tricula chiui"* to the hydrobiid genera *Oncomelania* and *Tricula*. As a result, I have found this snail to be another subspecies of *Oncomelania hupensis*, i.e., *O. hupensis chiui*. The purpose of this paper is to present the results of studies involving the anatomy, hybridization, and some biophysical properties of this subspecies. The systematics, phylogeny and importance of parasitological relationships are discussed.

MATERIALS AND METHODS

Methods involving the varied techniques used are discussed in each of the separate sections dealing with different aspects of this study.

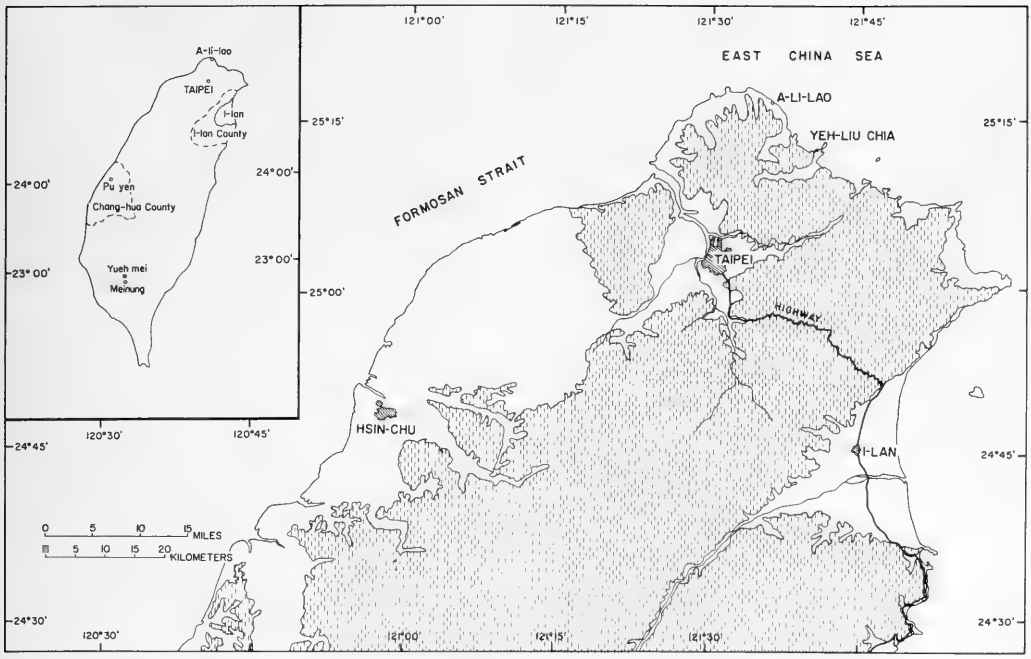


FIG. 1. Maps of Taiwan showing the localities for the populations studied in this paper. The type locality of *Oncomelania hupensis chiui* is at A-li-lao at the northern tip of the island. Shaded areas indicate mountainous regions.

Snails used in this study were *"Tricula chiui"* from the type locality at A-li-lao village in Taipei county, northern Taiwan, and, for comparison, various strains of *Oncomelania hupensis formosana* and other subspecies of *O. hupensis: O. h. quadrasi* came from Leyte, Philippines; *O. h. nosophora* was collected in Yamanashi Prefecture, Japan; progenitors of *O. h. hupensis* came from south China. In June, 1964, 2 populations of *O. h. formosana* were collected in I-lan county, north eastern Taiwan, and 1 population was collected from around Pu Yen village in Changhua county, in central western Taiwan (Fig. 1).

The collections were sent to the University of Michigan, Mollusk division, where they were placed in culture and used in electrophoretic and serological studies. Laboratory reared F_1 and F_2 generations, descended from the church population (Fig. 1) of I-lan snails, were also used for anatomical, electrophoretic and hybridization studies. In the immunological work, another strain of *Oncomelania hupensis formosana* was also used. These snails were descended from stocks (F_2 generation) that had been collected in Yueh Mei, southern Taiwan, by Dr. Kuntz in 1962. All anatomical studies were done at the 406th Medical Laboratory, Sagamihara City, Kanagawa Prefecture, Japan.

Habitat

From Figs. 1 and 2, it is evident that *Oncomelania hupensis chiui* is found on the coast of the northern tip of Taiwan. The habitat is exposed to winds off the sea laden with salt. The terrain is

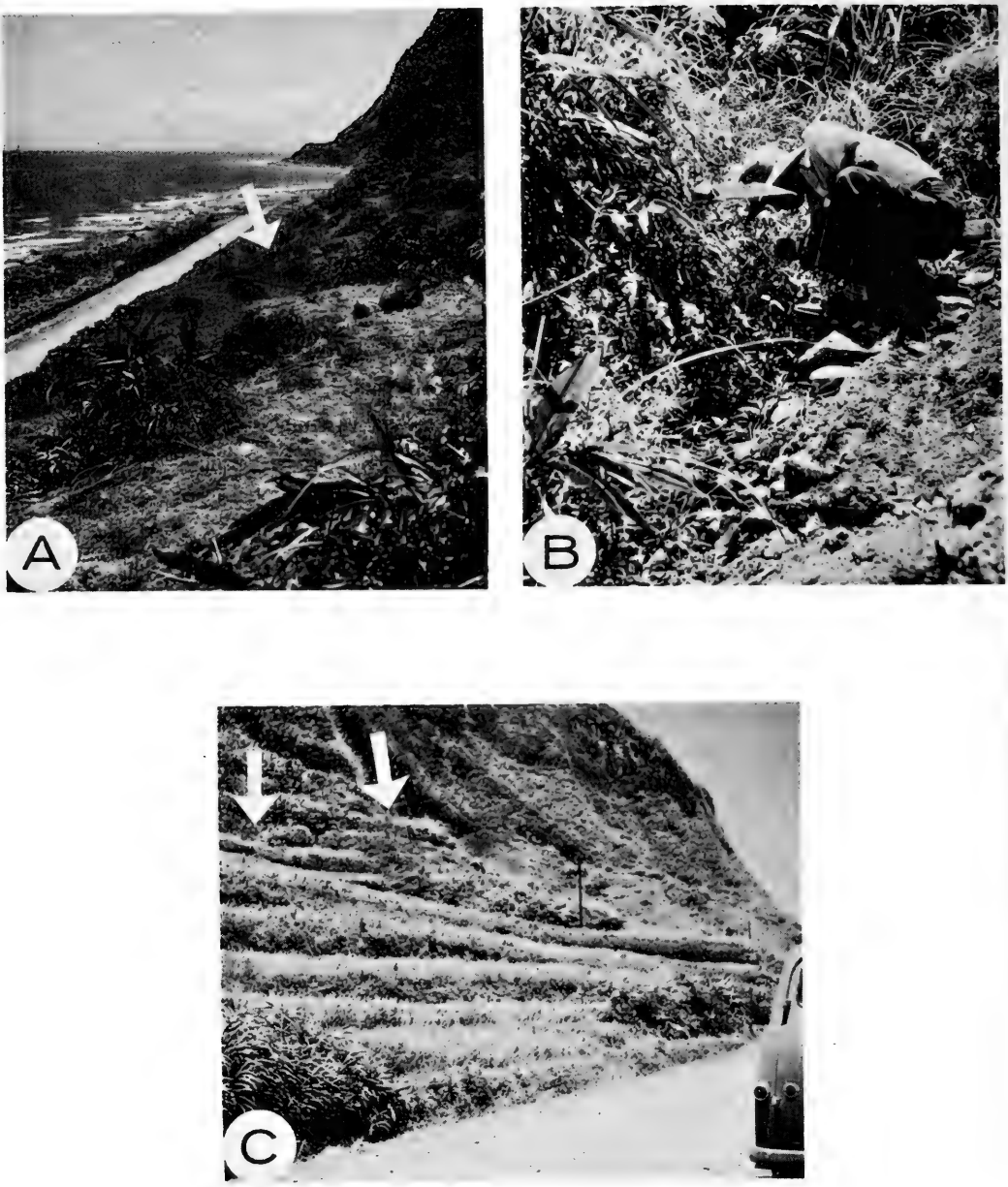


FIG. 2. Habitat of *Oncomelania hupensis chiui*.

A. General view showing the steep hilly nature of the habitat and its proximity to the sea.

B. Dr. Chiu peering into the small gully where water seeps downward in a shallow, gentle flow. The area is covered by dense vegetation.

C. The habitat is just above the terraced fields at the foot of the cliffs where the angle of the slope becomes quite steep.

rugged and the habitats are on steep slopes just where the slopes rise in abrupt cliffs (A, C, Fig. 2).

Snails were found in gullies eroded by channeled seepage and trickling water. The gullies (arrows, C, Fig. 2) are shallow, rocky and overgrown by dense brush. These amphibious snails are found submerged or out of water on rocks, leaves, sticks and small patches of soil. Dr Chiu is shown peering into one of the gullies (B, Fig. 2) which contains numerous snails of this subspecies.

This environment is in marked contrast to the usual habitats of the 4 previously known subspecies of *Oncomelania hupensis*, which inhabit the ditches around rice fields, irrigation canals and often the fields themselves, whereas the habitat of *O. h. chiui* is above the few terraced fields near the ocean and on steeper, more rugged terrain.

ANATOMY

1. Materials and Methods

Techniques used for dissections were described by Davis (1967). An additional technique was used in studying the nerves associated with the visceral ganglion. The uncoiled living snail was stained with Evan's blue dye (1:1000 aqueous) for 5-10 minutes. The result was a blue-stained epithelium with nerve tracts standing out white beneath the thin, ventral epithelium enclosing the mantle cavity.

Throughout the presentation of anatomical data, comparisons are made with *Oncomelania hupensis formosana* and *Pomatiopsis lapidaria* as given by Davis (1967). The latter snail serves for comparison with a genus of the Pomatiopsinae that is closely related to *Oncomelania* but qualitatively different in many aspects of anatomy. The orientation of organs and systems in the figures of this paper corresponds to that of the earlier study (Davis, 1967). All illustrations of anatomy were made by the author.

2. Shell

Habe & Miyazaki (1962) described the shell as "small, measuring about 4.5 mm in height, broadly ovate, rather solid, colored yellowish brown to brown. Nuclear whorls about two in number, smooth and polished, but usually eroded in the adult specimens. Post-nuclear whorls about 5, moderately inflated. Suture well impressed. Surface sculptured with numerous growth lines. Body whorl large and rounded, taking about two-thirds of the height of shell. Periphery obtusely angular in the young specimens, but well rounded in the fully grown specimens. Aperture rather large, roundly ovate, somewhat oblique, and slightly expanded. Peristome continuous, adnate at the parietal wall and colored dark brown at the edge. Outer lip short, arcuate and the columella short and weakly arched. Umbilical chink narrowed by the dilation of the columellar lip."

They figured the type and a paratype. Their material had 4.5-5.0 whorls, an average length and width of 4.46 and 2.73 mm respectively, and a ratio of length to width (L/W) of 1.63. Characteristic for these field specimens was the lack of any varix, i.e., the outer lip was thin and sharp. It was noted, however, that the umbilicus was more pronounced that the mere "chink" typical for several species of genuine *Tricula*.

In Fig. 3 are photographs of 3 topotypes collected by Robert E. Kuntz in 1961. The top 2 whorls in the population of 50 adults in that collection were without fail eroded and shells showed signs of weathering. Shells from that and later collections averaged 4.5-5 whorls, with an average length and width of 4.34 mm and 2.64 mm respectively. The L/W ratio was 1.64.

Snails were maintained in the laboratory as prescribed by Davis (1967) and van der Schalie & Davis (1968). Young were reared according to methods of van der Schalie & Davis (1965). Three laboratory reared snails are shown in

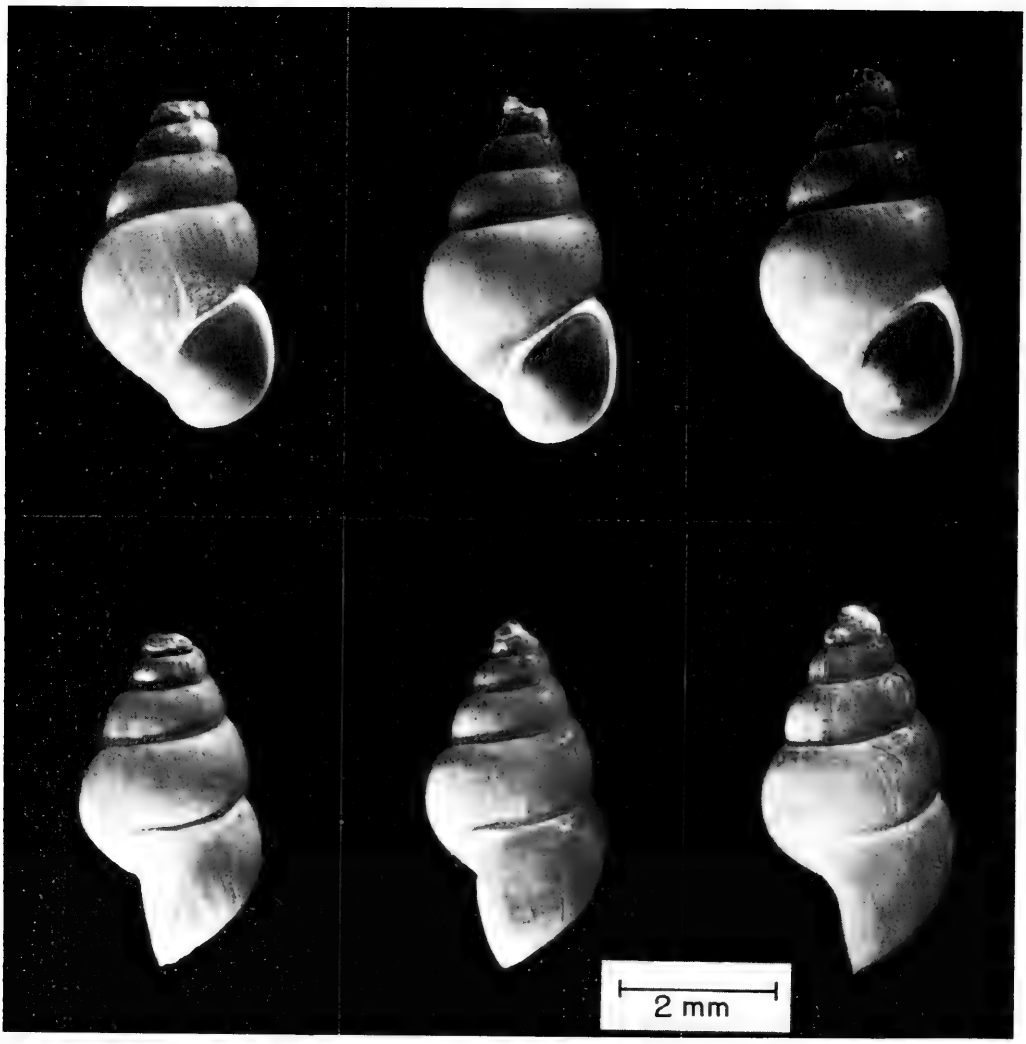


FIG. 3. Field collected *Oncomelania hupensis chiui*. Note sharp outer lip without varix and the eroded apex.

Fig. 4. Statistics pertaining to the shell are given in Table 1.

In Petri dish cultures snails reached adult size in 8 weeks, and did not grow any further when maintained singly beyond 9 weeks. There was a clear sexual dimorphism in whorl count and length. Males attained 6.0 whorls and averaged 4.36 mm in length while females with 6.5 whorls were larger, averaging 4.73 mm.

Perfect shells with complete spire indeed look like members of the *Oncomelania* complex. In Table 2 are given statistics on shell measurements for F_1 laboratory reared *O. h. formosana*, *O. h. chiui* and *O. h. quadrasi*. The data for *O. h. formosana* were taken from Davis (1967). All snails were reared in the same manner. Except for being wider at the base, the shell of *O. h. chiui* more closely resembles that of *O. h. quadrasi*.

Abbott (1948) says of the latter sub-

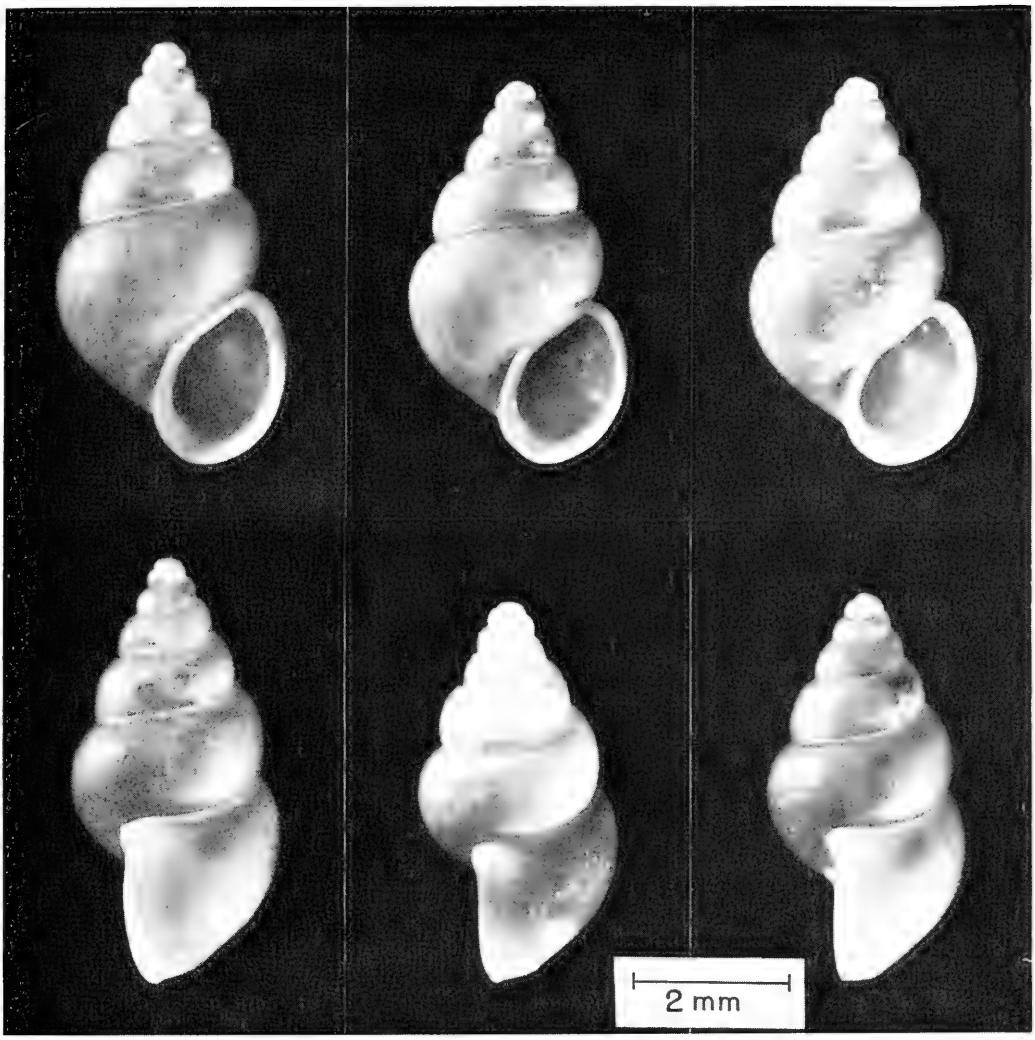


FIG. 4. First generation laboratory reared *Oncomelania hupensis chiui*. Note the perfect apices and the very slight thickening at the outer lip.

species that the adult shell "is from 3-5 mm in length with 6 to 7 whorls." He further states that the varix is generally only slightly developed. In the populations of *O. h. quadrasi* I collected in the Philippines from Oriental Mindoro, Leyte and South Luzon, the character of the varix varied greatly from well developed to nearly absent. In the laboratory reared populations the varix was nearly always discernible in adults, but slightly less pronounced than that shown by Davis (1967) for *O. h. formosana*.

In laboratory reared *Oncomelania hupensis chiui*, the varix was faint. The lip was thickened, as shown in Fig. 4, but did not produce the marked outfolding of shell moderately developed in *O. h. quadrasi* or pronounced in *O. h. formosana*. The point to be made is that under optimal conditions the lip was not simple, thin and sharp as in the field snails, but had a faint varix.

TABLE 1. Various parameters measured on 9 adult shells of *Oncomelania hupensis chiui* reared in the laboratory

Feature measured (mm) or counted	Sex	Statistics		
		$\bar{X}$	S	Se
Number of whorls	♂	6.0	-	-
	♀	6.5	-	-
		mm	mm	mm
Length shell	♂	4.36	0.09	0.03
	♀	4.73	0.15	0.05
Width shell	♂	2.47	0.07	0.02
	♀	2.74	0.12	0.04
Length parietal callus		0.94	0.02	0.006
Length aperture		1.89	0.12	0.04
Apical whorl:				
Width		0.35	0.02 (t.v.)	
Width of tip		0.14	0.02 (t.v.)	

$\bar{X}$ = Mean
S = Standard deviation
Se = Standard error of the mean
t.v. = total variation

TABLE 2. Comparison of adult shell[2] dimensions of 3 subspecies of *Oncomelania hupensis*

Shell feature measured (mm) or counted	Taxa		
	chiui (9)	*quadrasi* (25)	*formosana* (25)
Greatest number of whorls	6.5	6.5	7.0 - 7.5
Length of shell	4.73	4.79	6.30
Width of shell	2.74	2.62	3.00
Ratio L/W	1.73	1.83	2.10
Length aperture	1.89	2.04	2.40
Length parietal callus	0.94	0.94	1.08
Width apical whorl	0.35	0.35	0.34
Width of tip of apical whorl	0.14	0.14	0.12

[2] Shells were picked at random from snails having the greatest whorl count and in which varix formation (*O. h. quadrasi, O. h. formosana*) or a thickened outer lip (*O. h. chiui*) were observed.

As discussed and illustrated by Davis (1967) there is a sinuation of the outer lip in *Oncomelania hupensis formosana*. In field *O. h. chiui* (Fig. 3) the lip is straight or shows only the slightest sinuation. The laboratory reared snails Fig. 4) showed the more typical sinuation found in *O. h. formosana* and *O. h. quadrasi*.

On the basis of shell, it is concluded that the snail under consideration belongs in the *Oncomelania* complex. The

only difference is the very slight development of varix.

3. External Morphology and Topography

External features of the head area and mode of progression clearly indicate that *Oncomelania hupensis chiui* is a member of the Hydrobiidae, subfamily Pomatiopsinae.

The pedal crease is evident in the step-like progression of the animal. The suprapedal fold is present, as is the omniphoric groove (previously discussed by Davis, 1967). The head area is evenly dusted with grey pigment.

With the animal moving about under water the extended tentacles were 1.00-1.09 mm long in the adults, measurements comparing well enough with the length range of 0.96-1.20 mm for *Oncomelania hupensis formosana* (Davis, 1967). The tentacles bear eyes in pronounced swellings at their outer bases. The mid-lateral border of the eyes is encircled by white granules which, in a few cases, extend in front of the eyes and a very short way out on the tentacles. These "eyebrows" are white, not yellow as in *O. h. quadrasi* or pale yellow to white-yellow as in *O. h. formosana.*

Sexual dimorphism is evident in the pigment pattern of the animal, as was the case in *Oncomelania hupensis formosana.* In the apical body whorls of males, the dorsal surface of the stomach and digestive gland is covered with a uniform dark pigment (Pi; Fig. 7A). About 20% of the laboratory reared female snails had only a slight amount of pigment, in a narrow strip 0.063 mm wide near the apex. In field collected females pigment was frequently found lightly dusting the stomach. About 2/3 of the digestive gland was devoid of pigment but at the tip a narrow strip often traversed the mid-dorsal region of the gland.

The arrangement of organs is exactly as described for *Oncomelania hupensis formosana* (Davis, 1967). The relative sizes of digestive gland and total body length for *O. h. chiui*, *O. h. formosana* and *Pomatiopsis lapidaria* are shown in Table 3. *O. h. chiui* is clearly an order of magnitude smaller than *O. h. formosana.*

4. Mantle Cavity

In field snails there were 32-36 ctenidial filaments (without correlation to sex). Laboratory reared adult males had 26+ 5 gill filaments while females had 32+ 4. These data differ considerably from those of Habe & Miyazaki (1962) who state that "gill lamellae moderately developed, about 20 in number."

The length of the row of gill filaments is similar in the 2 subspecies of *Oncomelania hupensis* and distinctly greater than in *Pomatiopsis lapidaria* (Table 3).

The relationship of the osphradium to the gills and to the anterior end of the mantle cavity is shown in Fig. 10. The osphradial ganglion (Og) is clearly discernible within the osphradial pit (Opi). The positional relationship between osphradium and ctenidium is the same in both *Oncomelania hupensis formosana* and *O. h. chiui*, although the osphradium is somewhat larger in the latter (Table 3).

Organs within the mantle cavity are the same as those discussed in detail by Davis (1967).

5. Female Reproduction System (Figs. 5, 6)

The female reproductive system is similar in structure and position of organs to that in *Oncomelania hupensis formosana.* The uncoiled female is shown in Fig. 5.

The oviduct (Ov) is shown broken, due to uncoiling the body. The gonopericardial duct (not figured) was found leading into the kidney tissue (Ki) from the dorsal side of the oviduct where it turns medially.

Gonad. The gonad (Go; Figs. 5; 6A, D-F) is multibranched (3-5 branches). It is distinctly shorter than that of *Oncomelania hupensis formosana* and *Pomatiopsis lapidaria*, but has

TABLE 3. Comparison of organ size (mm), in forms of *Oncomelania hupensis* and *Pomatiopis* exclusive of the nervous system[3]

Organ	Dimension	Sex	Taxa		
			O. h. chiui mm	*O. h. formosana* mm	*P. lapidaria* mm
1. Tentacles	L	-	1.00-1.09	0.96-1.20	0.60-0.90
2. Digestive gland	L	♀	2.66-2.90	3.23*	4.78*
	W (at stomach)	♀	0.73-0.89	1.40*	1.07*
3. Total body	L	♀	7.4*	8.9*	10.6*
4. Gills	Number	♂	26 ± 5	46 ± 4	22 ± 2
		♀	32 ± 4		25 ± 3
	L	-	1.94 ± 0.24	1.91*	1.57*
5. Osphradium	L	-	0.63; S, 0.22	0.50 ± 0.09	0.59 ± 0.12
6. Buccal mass	L	-	0.720-0.780	0.720-0.860	0.850-1.080
7. Reproductive system		♀			
1) Gonad	L		0.79-1.23	1.12-1.61	1.10-1.61
2) Bursa copulatrix	L		0.73-0.92	0.74-0.94	0.58-0.86
	W		0.34-0.48	0.33-0.43	0.45-0.51
3) Seminal receptacle:					
Duct	L		0.24†	0.14-0.19	0.12-0.25
	W		0.03†	0.05†	0.06-0.15
Swollen portion	L		0.21†	0.17-0.31	0.20-0.24
	W		0.15†	0.12-0.24	0.17-0.24
4) Pallial oviduct	L		3.14 ± 0.20	3.36-4.00	4.30-5.00
	W (greatest)		0.60 ± 0.12	0.60*	0.72*
1) Prostate	L	♂	1.69-2.18	2.20-2.25	1.68-1.75
	W		0.68-0.73	0.62-0.72	0.70
2) Verge	L		2.18-2.66	3.36 ± 0.12	2.60-3.40
	W (base)		0.68-0.85	0.63*	0.63*

L = length
W = width
S = standard deviation
± = gives total variation

* = measurement from 1 adult individual
† = variability not encountered in measuring 6 individuals

[3] Measurements were taken from 6-12 adult individuals unless otherwise indicated. The total range is given without a mean value when only 6-7 measurements were made.

the same structure as *O. h. formosana*. Although *O. h. formosana* and *P. lapidaria* have the same size ovary, their structure is quite distinct and different (Davis, 1967).

Bursa copulatrix. The bursa (Bu) is shown from the ventral aspect in Figs. 5A; 6A, B. Its length and width in the 3 taxa are compared in Table 3. Size and shape in the 2 subspecies of *Oncomelania* are comparable.

The sperm duct (Sdu) and spermathecal

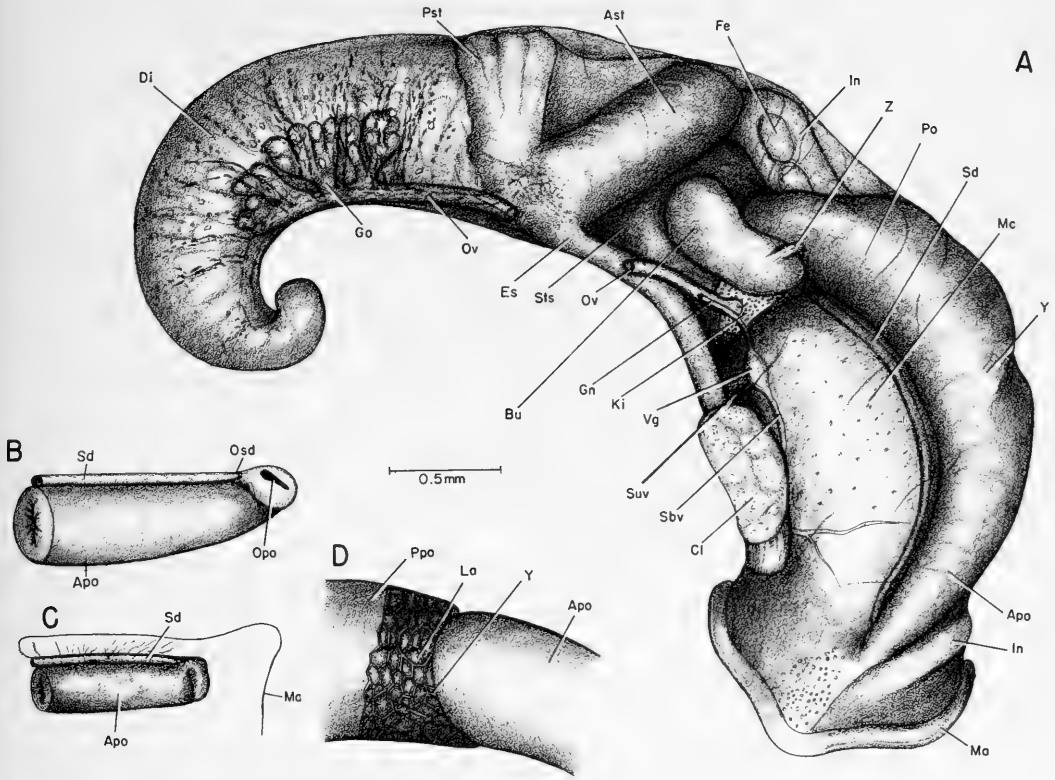


FIG. 5. Female reproductive system of *Oncomelania hupensis chiui*.

A. Uncoiled female showing the ventral or columellar side of the body. The kidney tissue was removed, in part, to reveal the entire bursa copulatrix (Bu); only the tissue encircling the oviduct near the end of the mantle cavity was not removed.

B. Anterior portion of the pallial oviduct (Apo) and spermathecal duct. The anterior end of the pallial oviduct has been bent towards the viewer to expose the opening.

C. Anterior portion of the pallial oviduct and spermathecal duct as normally viewed with the cut mantle folded back to expose this region.

D. Region (Y) where the posterior and anterior portions of the pallial oviduct join. The network of connective tissue chambers seen beneath the epithelium of the posterior section is partially indicated.

Apo	anterior section of the pollial oviduct
Ast	anterior chamber of the stomach
Bu	bursa copulatrix
Cl	columellar muscle
Di	disgestive gland
Es	esophagus
Fe	fecal pellet
Go	gonad
Gn	gonadal nerve
In	intestine
Ki	kidney
La	a section of the lattice-work of connective tissue enclosing the glandular units found throughout the posterior portion of the pallial oviduct
Ma	mantle edge
Mc	epithelium covering the mantle cavity
Opo	opening of the pallial oviduct
Osd	opening of the spermathecal duct
Ov	oviduct
Po	pallial oviduct
Ppo	posterior portion of the pallial oviduct
Pst	posterior chamber of the stomach
Sbv	subvisceral connective
Sd	spermathecal duct
Sts	style sac
Suv	supravisceral connective
Vg	visceral ganglion
y	point where the anterior and posterior sections of the pallial oviduct join
z	ducts leaving the bursa copulatrix

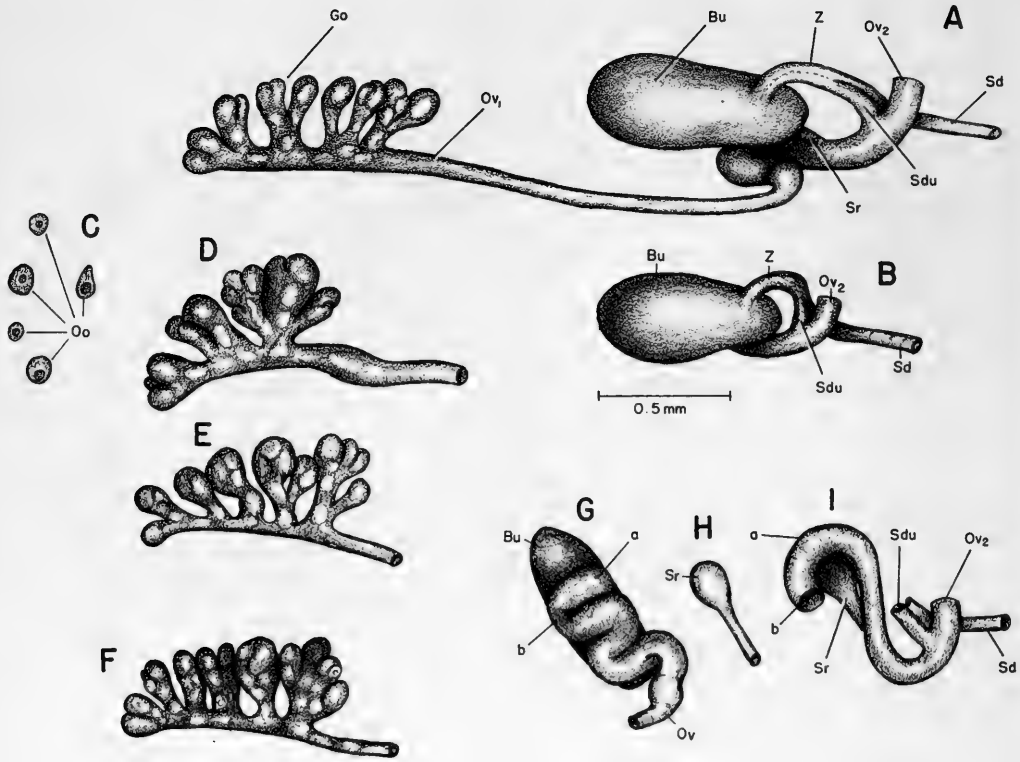


FIG. 6. Female reproductive system of *Oncomelania hupensis chiui*.

A. Arrangement of ducts associated with the gonad and bursa copulatrix, before the oviduct enters the pallial oviduct. Dashed line shows that the tubes (spermathecal and sperm ducts) leaving the bursa are separate but enclosed in a common connective tissue sheath.

B. Variation in shape of bursa copulatrix and the ducts leaving the bursa, as seen in gross dissection.

C. Oocytes of various sizes and shapes as dissected from the gonad.

D–F. Variation in branching and structure of the gonad.

G. The bursa rotated to show the dorsal surface with coiled oviduct. The coils occlude the seminal receptacle.

H. Seminal receptacle.

I. A portion of the coiled oviduct removed to show relationship of seminal receptacle, oviduct and spermathecal duct.

a	tip of coil of the oviduct dorsal to the bursa copulatrix corresponding to the equivalent points in Figs. G and I	Ov_1	oviduct, from gonad to the sperm duct (Sdu)
b	point shown if Figs. G and I, cut in Fig. I to reveal the seminal receptacle (Sr)	Ov_2	oviduct, from sperm duct to entry into pallial oviduct
Bu	bursa copulatrix	Sd	spermathecal duct
Go	gonad	Sdu	sperm duct
Oo	oocytes	Sr	seminal receptacle
		z	ducts leaving the bursa copulatrix in common sheath

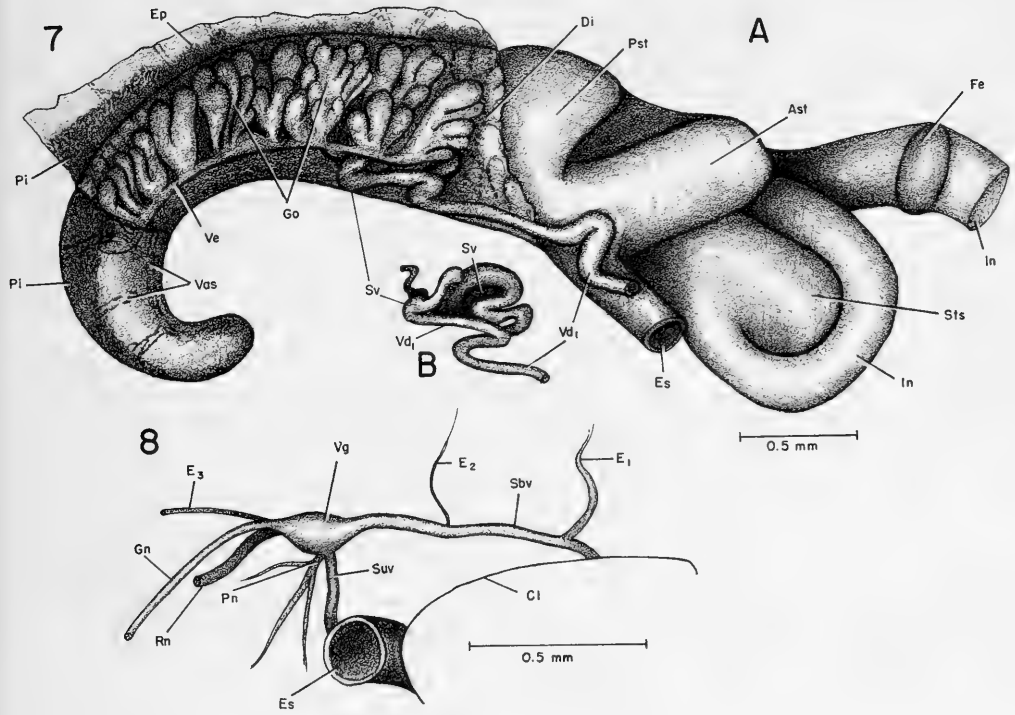


FIG. 7. Male reproductive system of *Oncomelania hupensis chiui*.

A. Uncoiled mid- and posterior body of a male, ventral view. The digestive gland abuts on the stomach. The connective and kidney tissues were removed to show the exterior ventral structure of the stomach.

B. Coiled section of the vas deferens (seminal vesicle) which is covered in Fig. A by the anterior section of the gonad.

FIG. 8. The visceral ganglion complex showing an unusual variation in the position where the pericardial nerve arises. The nerve (Pn) usually arises from the supravisceral connective (Suv) slightly removed from the visceral ganglion (Vg) as shown in Fig. 12.

Ast	anterior chamber of the stomach	Pi	pigment
Cl	columellar muscle	Pn	pericardial nerve
Di	digestive gland	Pst	posterior chamber of the stomach
E_1	external mantle cavity nerve 1	Rn	renal nerve
E_2	external mantle cavity nerve 2	Sbv	subvisceral connective
E_3	external mantle cavity nerve 3	Sts	style sac
Es	esophagus	Suv	supravisceral connective
Fe	fecal pellet	Sv	seminal vesicle
Ep	epithelium	Vd_1	posterior section of vas deferens
Gn	gonadal nerve	Vas	vascular elements and visceral artery
Go	gonad	Ve	vas efferens
In	intestine	Vg	visceral ganglion

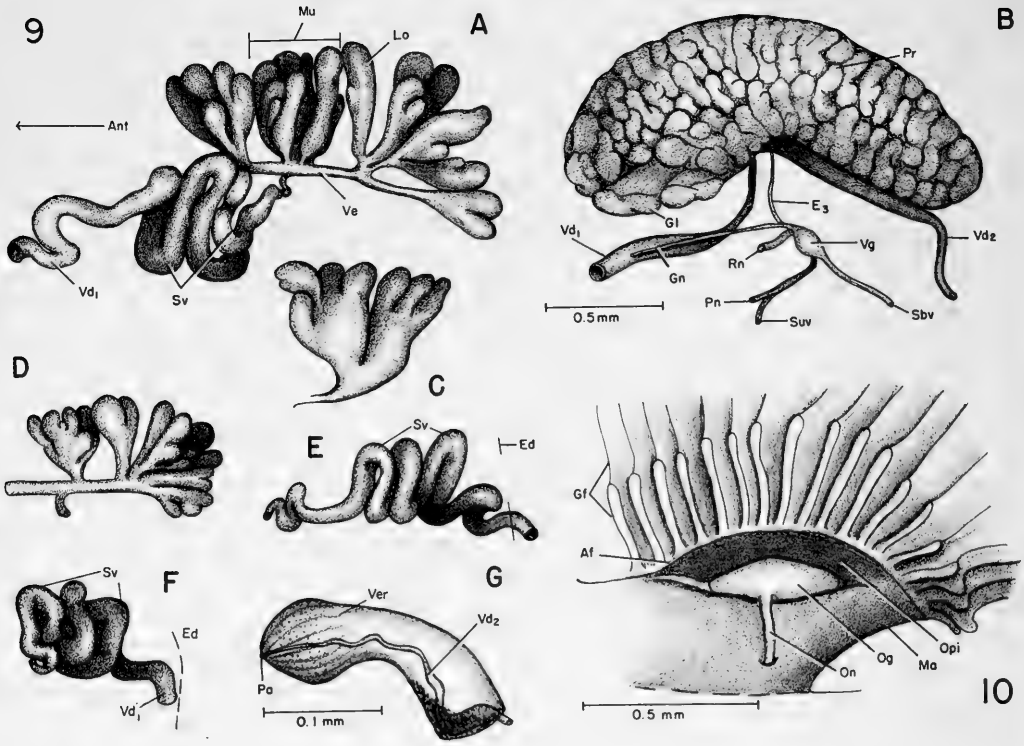


FIG. 9. Male reproductive system of *Oncomelania hupensis chiui*. Figs. A-F are at the same scale.

A. Dorsal aspect of the gonad with the anterior multibranched units (Mu) removed. The seminal vesicle, and the point of connection with the vas efferns, are shown. In this specimen this connection was in an unusual position, being slightly posterior to mid-gonad. Generally, one observes more individual lobes (Lo) and how they arise and branch, from the dorsal aspect than from the ventral (compare with Figs. 7A and 9D).

B. Ventral view of prostate showing relationship to anterior and posterior vas deferens and visceral ganglionic complex.

C. A single lobe from a multibranched testicular unit viewed posteriorly. It represents the width of the gonad.

D. Ventral aspect of several multibranched units at the anterior end of the gonad from a different specimen than the one used for Fig. 9A.

E-F. Variation in coiling of the seminal vesicle. Dashed line represents anterior end of the digestive gland.

G. The verge.

FIG. 10. Relationship of ctenidium and osphradium. The posterior gill filaments are not shown.

Af	afferent vessel	Gn	gonadal nerve
Ant	anterior direction	Lo	individual testicular lobe from a multi-branched gonodal unit
Ed	anterior end of digestive gland		
E_3	external mantle cavity nerve 3	Ma	anterior mantle, edge of the reflected mantle
Gf	Gill filament		
Gl	individual glandular unit of the prostate	Mu	one multibranched gonodal unit

duct (Sd) arise from the bursa just as they do in *Oncomelania hupensis formosana*, bound together by connective tissue so as to appear as 1 tube (z; Figs. 5; 6A, B). The dotted line in Fig. 6A represents the actual internal division of those tubes. As in *O. h. formosana*, these ducts are overgrown by tissue of the pallial oviduct (Po; Fig. 5A). The area just anterior to the bursa (Fig. 5A) is further occluded by kidney tissue (Ki) and connective tissue. When the overlying tissues are removed, the relationship of the tubes becomes clear (Fig. 6A, B). The sperm duct (Sdu) and the spermathecal duct (Sd) diverge, the former bending to join the oviduct (Ov_2, Fig. 6A, B), the latter passing medially and dorsal to the oviduct just where the oviduct enters the pallial oviduct to continue along the mantel cavity.

Seminal receptacle. When the bursa copulatrix (Bu) as shown in Fig. 6A is turned over 180° (Fig. 6G) one observes the dorsal convoluted section of the oviduct. The coils are pressed against the bursa copulatrix hiding from view the seminal receptacle situated between them. Removal of part of the coil (Fig. 6I) shows that the oviduct coils around the seminal receptacle (Sr). The arrangement of this coiled section of oviduct and the manner in which it encircles the seminal receptacle are exactly as found in *O. h. formosana*.

The seminal receptacle is similar in shape to that of *O. h. formosana* (Sr; Fig. 6H). It is compared with this organ in the other taxa in Table 3. The duct from the swollen portion of the receptacle to the oviduct is noticeably very narrow (0.03 mm wide). As shown in Fig. 6A, the duct (Sr) enters the oviduct close to the opening of the sperm duct (Sdu). Most frequently this point is occluded by the anterior edge of the bursa (Fig. 6B).

The swollen receptacle is bound to the bursa by distinct connective tissue in which are imbedded numerous white granules.

Pallial oviduct. The pallial oviduct is a huge organ which overlies the organs at the mid-body region, the posterior end of the mantle cavity and the anterior mantel cavity (Po; Fig. 5A). The organ is clearly separable into 2 sections when viewed at 16X-40X under direct illumination; a grey-white posterior section (Po) and a white anterior section (Apo). The dividing point is shown in Fig. 5A by the pronounced dip (at y) which occurs at the outer curvature about mid gland. The sections differ in texture; the posterior section appears more glandular, the epithelium is stretched by loose swollen glandular pockets bounded by a definite honeycomb-like framework of connective tissue (La; Fig. 5D). The anterior section is more slender and composed of solid white tissue lacking the glandular macroscopic units.

The length and greatest width of the organ are compared with that found in other species in Table 3. It is not as long as in *Oncomelania hupensis formosana*, but is as wide at the posterior end.

Cutting the mantle between the columellar muscle (Cl) and spermathecal duct (Sd; snail oriented as in Fig 5A) and folding back the mantle, exposes the anterior end of the pallial oviduct and

---

Og osphradial ganglion
On osphradial nerve
Opi osphradial pit
Pa papilla
Pn pericardial nerve
Pr prostate
Rn renal nerve
Sbv subvisceral connective
Suv supravisceral connective
Sv seminal vesicle
Vd_1 vas deferens from gonad to prostate
Vd_2 pallial vas deferens; from prostate to tip of verge
Ve vas efferens
Ver verge
Vg visceral ganglion

spermathecal duct (Fig. 5C). As shown in that figure, the thickened lip at the anterior end of the pallial oviduct (Apo) presses over its opening. When the end of the organ is pulled upward and bent slightly toward the viewer the opening is seen (Opo; Fig. 5B).

The spermathecal duct terminates, as shown (Osd; Fig. 5B), before reaching the thickened lips of the terminal pallial oviduct. The relationship of these organs to each other and the mantle cavity is the same in *Oncomelania hupensis formosana* and is more fully discussed by Davis (1967) for that taxon.

6. Male Reproductive System (Figs. 7, 9)

This system corresponds in organ structure and position to that in *Oncomelania hupensis formosana* (Davis, 1967).

Gonad. The posterior section of the body tube is shown uncoiled in Fig. 7A. The digestive gland (Di) abuts on the posterior section of the stomach (Pst). A strip of epithelium (Ep) is pulled back to clearly show the gonad (Go) which lies just beneath the epithelium.

From 7-9 multibranched units (Mu; Fig. 9A) arise from a slender vas efferens (Ve). The vas deferens (Vd_1) usually arises from the anterior 1/3 of the vas efferens. The drawings in Figs. 7A, 9A and 9D were made using different specimens. The points where the lobes (Lo) arise from the vas efferens or from a common duct arising from the vas efferens are generally more clearly seen from the dorsal aspect (Fig. 9A). In ventral view (Figs. 7A and 9D) the basal portions of a greater number of lobes are obscure. Figs. 9A, D show the relationship of the lobes in the multibranched units and how the units arise from the vas efferens. Fig. 9C shows the width of the gonad as composed of 2 lobes.

Seminal vesicle. The vas deferens becomes a spherical knotted mass of tubes (Sv) soon after leaving the vas efferens (Figs. 7B, 9A). This confusing knot of tubes is called the seminal vesicle. As viewed and shown in Fig. 7A, the seminal vesicle is hidden beneath the anterior 1/3 of the gonad. Only the lateral edge is showing where the vas deferens (Vd_1) leaves the coil and runs toward the stomach. With the gonadal lobes removed, the seminal vesicle is exposed (Fig. 7B). Variations in coiling are shown in Fig. 9A, E, F. The dashed line (Ed) in the figures indicates the anterior end of the digestive gland.

Prostate. The prostate (Fig. 9B) occupies the same position as the pallial oviduct in the female but is not as long. It overlaps the posterior end of the mantle cavity.

The glandular nature of the ventral prostate surface is shown in Fig. 9B. The length and greatest width (posterior end) of the prostate are compared with that of the other taxa in Table 3. Greater detail of the anatomy of the prostate and how the posterior vas deferens (Vd_1) and pallial vas deferens (Vd_2) connect with it, is given by Davis (1967) for *Oncomelania hupensis formosana,* which has the same prostate anatomy.

Verge. The verge (penis) is shown in Fig. 9G. It does not differ from that of the other subspecies of *Oncomelania.* The anterior end is muscularly thickened. In this area longitudinal muscle strands are evident. There is a protrudable papilla at the tip of the verge which is otherwise blunt and flattened.

The verge is ciliated exactly as described for *Oncomelania hupensis formosana* (Davis, 1967). Likewise, the glandular units correspond. The length of verge and width at the base are compared for the 3 snails in Table 3.

7. Muscular system

The only portion of the muscular system illustrated or discussed here pertains to the exterior buccal mass (Fig. 11A). The musculature, as a whole, is that described for *Oncomelania hupensis formosana* and is similarly labeled (Davis, 1967). Not shown in Fig. 11A are suspensors of the buccal

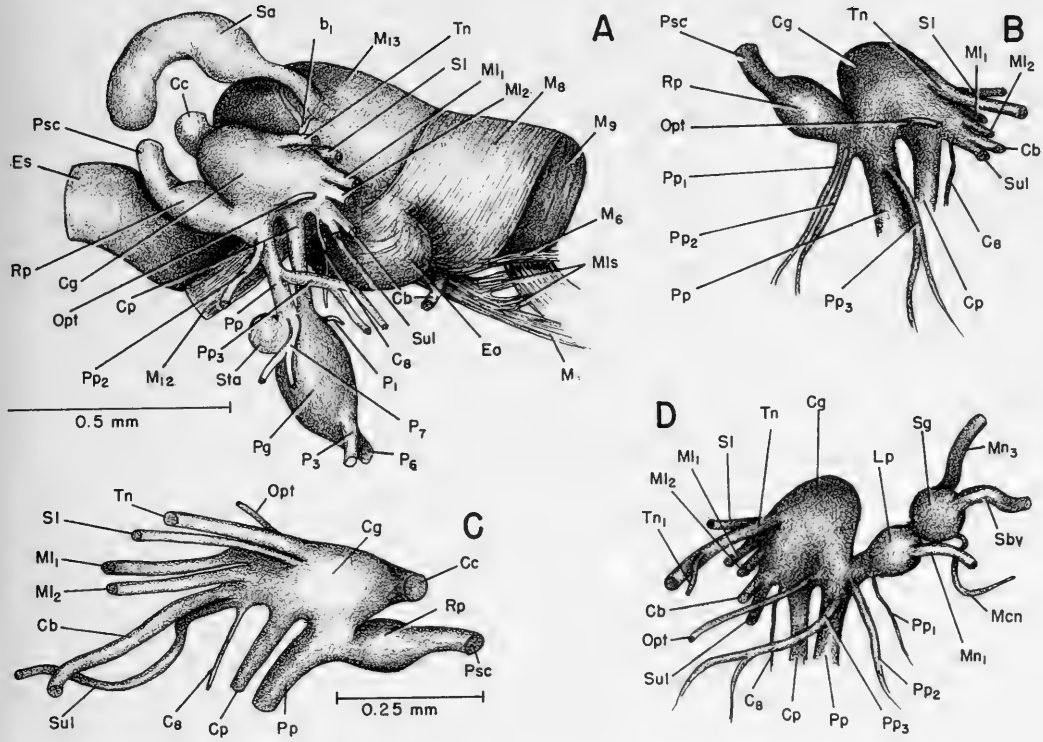


FIG. 11. Nervous and muscular system of *Oncomelania hupensis chiui*.

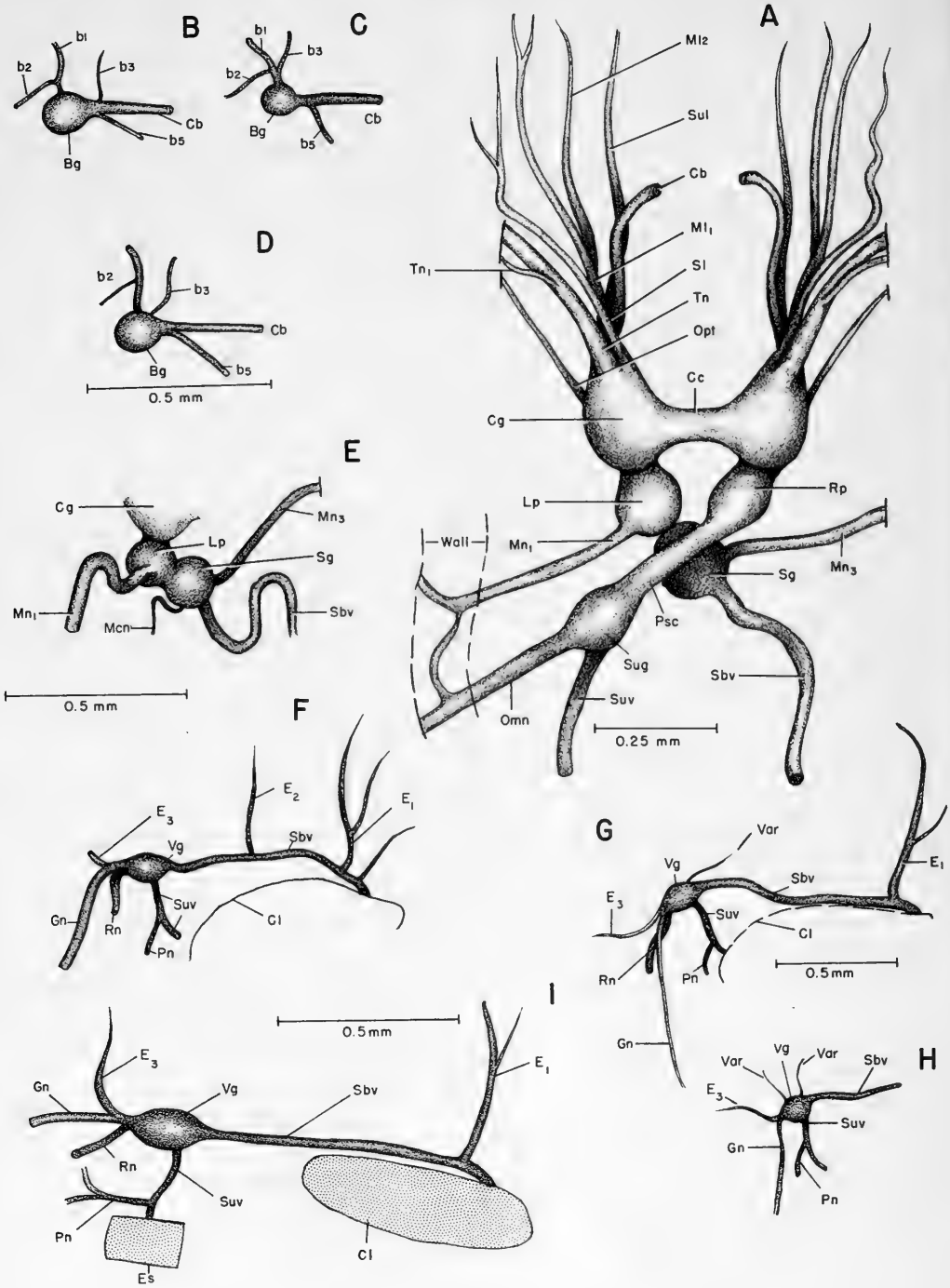
A. Right lateral aspect of buccal mass, cerebral and pedal ganglia.

B. Right cerebral and pleural ganglia showing variation in emergent nerves.

C. Medial aspect of right cerebral ganglion showing where the nerves arise.

D. Left cerebral ganglion, pleural ganglion, and subesophageal ganglion.

b_1	dorsal buccal nerve
Cb	cerebro-buccal connective
Cc	cerebral commissure
Cg	cerebral ganglion
Cp	cerebro-pedal connective
C_8	cerebro-tensor nerve
Eo	external odontophore membrane
Es	esophagus
Lp	left pleural ganglion
M_5	buccal protractor muscle
M_6	preventral protractor
M_8	anterior jugalis
M_9	buccal constrictor
M_{12}	buccal retractor
M_{13}	membranous jugalis
Mcn	midcolumellar nerve
Ml_1	median labial nerve 1
Ml_2	median labial nerve 2
Mls	medio-lateral slips of buccal protractor
Mn_1	mantle nerve 1
Mn_3	mantle nerve 3
Opt	optic nerve
P_1	lateral retractor nerve
P_3	major lateral nerve
P_6	metapodial connective
P_7	dorso-lateral pedal nerve
Pg	pedal ganglion
Pp	pleuro-pedal connective
Pp_1	lateral nerve 1
Pp_2	penial nerve
Pp_3	lateral nerve 3
Psc	pleuro-supraesophageal connective
Rp	right pleural ganglion
Sa	salivary gland
Sbv	subvisceral connective
Sg	subesophageal ganglion
Sl	supralabial nerve
Sta	statocyst
Sul	sublabial nerve
Tn	tentacular nerve
Tn_1	branch, tentacular nerve


A
Mi2
Sul
Cb
Mi1
Tn1
Sl
Tn
Opt
Cc
Cg
Lp
Rp
Wall
Mn1
Mn3
Sg
Psc
Sug
Sbv
Suv
Omn
0.25 mm
B
b1
b2
b3
Cb
b5
Bg
C
b1
b3
b2
Cb
Bg
b5
D
b2
b3
Cb
Bg
b5
0.5 mm
E
Cg
Mn3
Lp
Sg
Mn1
Mcn
Sbv
0.5 mm
F
E2
E1
E3
Vg
Sbv
Suv
Gn
Rn
Cl
Pn
G
Var
Vg
E1
Sbv
E3
Suv
Cl
0.5mm
Rn
Pn
Gn
H
Var
Vg
Var
Sbv
E3
Suv
Gn
Pn
I
0.5mm
E3
E1
Gn
Vg
Sbv
Rn
Suv
Pn
Cl
Es

mass and the preventral dilators. Both groups are present; the former running between the muscles M_8, M_9 and the roof of the cephalic haemocoel, the latter from M_9 to the lateral floor of the cephalic haemocoel.

The well defined medio-lateral slips (Mls) of the buccal protractor (M_5) differed slightly from their counterpart in *O. h. formosana*. In the latter subspecies the buccal protractor has 2 slips which are either united in a single sheet or, more frequently, split. When split, one slip originates on the rostral retractor, the other on the anterior ventro-lateral rostral wall. In *O. h. chiui* the most common arrangement consists of 2 slips as described for the above subspecies. However, 3 slips are frequently encountered, in which case 2 distinct bands instead of 1 (medio-lateral slips of the buccal protractor, Mls, Fig. 11) originate from the rostral retractor or the oral sphincter (anterior, ventral rostral region) and insert on the main band of the buccal protractor (M_5, Fig. 11).

8. Nervous System (Figs. 5, 8-13)

The nervous system was dissected with the following in mind: 1) to present all nerves arising from each ganglion as they actually appeared; 2) define limits of variation in these main nerves in terms of position and number; 3) compare the above with *Oncomelania hupensis formosana*. Orientation in the illustrations and terminology are the same as presented for *O. h. formosana* (Davis, 1967).

A. Cerebral complex

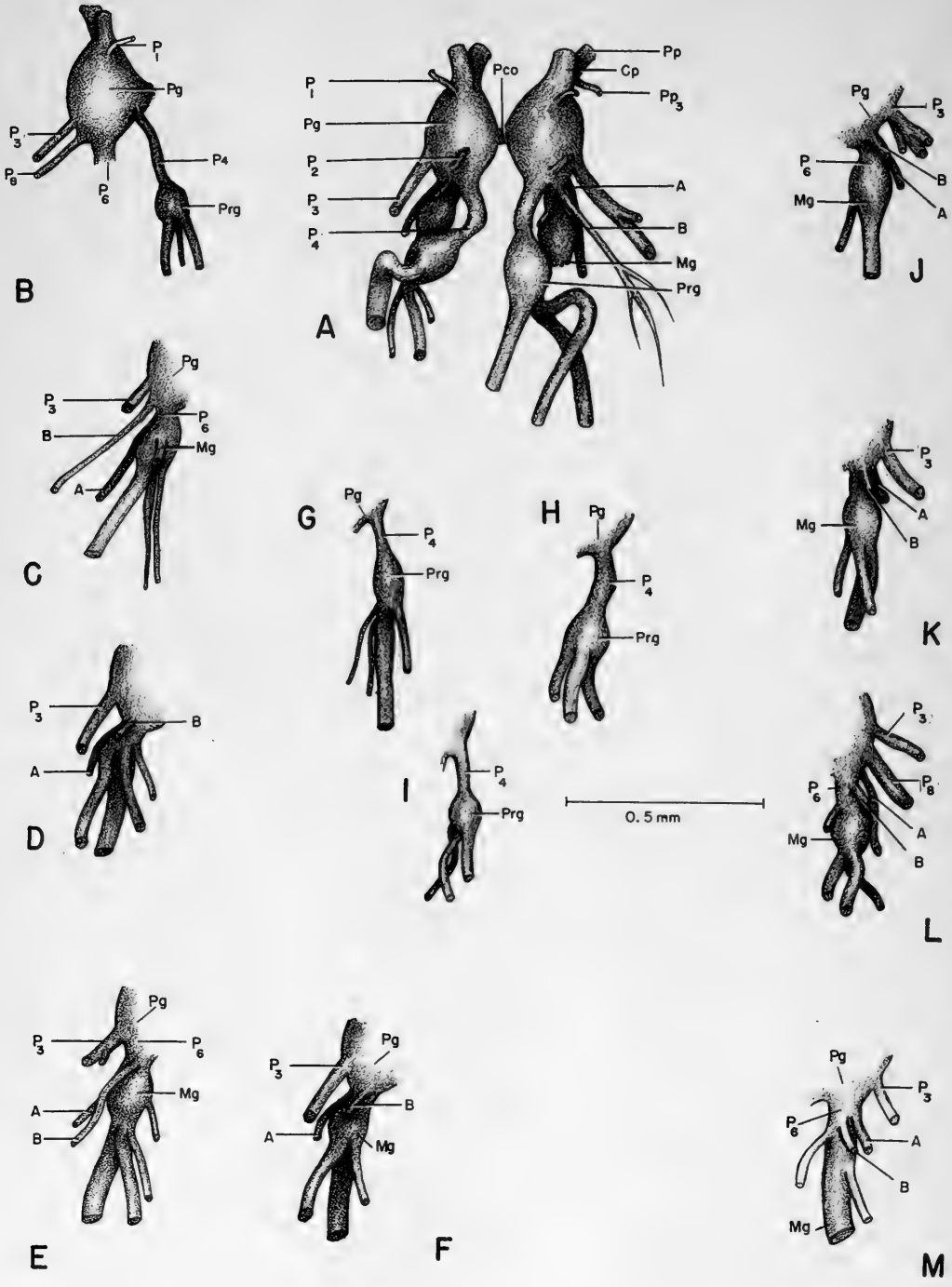
(1) Dorsal Aspect: The relationship of the cerebral ganglia to the buccal mass and esophagus is shown in lateral view in Fig. 11A. The dorsal aspect of the cerebral ganglia is shown in Fig. 12A. When the dorsal mid-line of the rostrum is opened, only the following nerves are observed: 1) optic (Opt), 2) tentacular (Tn), 3) supralabial (Sl), and 4) sometimes median labial 1 (Ml_1).

Dimensions of cerebral ganglion, cerebral commissure (Cc), and combined width of cerebral ganglia plus commissure are given in Table 4 in comparison with *Oncomelania hupensis formosana* and *Pomatiopsis lapidara*.

The only detectable differences be-

FIG. 12. Nervous system of *Oncomelania hupensis chiui*.
A. Dorsal aspect of central nervous system.
B-D. Variations in buccal ganglion complex.
E. Left pleural and subesophageal ganglia underlying the pleurosupraesophageal connective in Fig. A.
F-I. Variations in visceral ganglion complex.

b_1	dorsal buccal nerve	Mn_3	mantle nerve 3
b_2	esophageal nerve	Omn	osphradiomantle nerve
b_3	central buccal nerve	Opt	optic nerve
b_5	odontophoral nerve	Pn	pericardial nerve
Bg	buccal ganglion	Psc	pleuro-supraesophageal connective
Cb	cerebro-buccal connective	Rn	renal nerve
Cc	cerebral commissure	Rp	right pleural ganglion
Cg	cerebral ganglion	Sbv	subvisceral connective
Cl	columellar muscle	Sg	subesophageal ganglion
E_1	external mantle cavity nerve 1	Sl	supralabial nerve
E_2	external mantle cavity nerve 2	Sug	supraesophageal ganglion
E_3	external mantle cavity nerve 3	Sul	sublabial nerve
Gn	gonadal nerve	Suv	supravisceral connective
Lp	left pleural ganglion	Tn	tentacular nerve
Mcn	midcolumellar nerve	Tn_1	branch, tentacular nerve
Ml_1	median labial nerve 1	Var	variant nerves
Ml_2	median labial nerve 2	Vg	visceral ganglion
Mn_1	mantle nerve 1	Wall	left cephalic wall


P1
Pg
P3
P4
P2
P6
Prg
B
Pco
Pp
Cp
Pp3
A
Mg
J
P5
P7
C
D
G
H
I
K
L
E
F
M
0.5 mm

tween the 2 subspecies of *Oncomelania hupensis* in dorsal view were: 1) generally, a "bulb" was lacking at the base of the tentacular nerve in *O. h. chiui;* it was present, though weak, in a few specimens only. Such a bulb is strongly developed in *O. h. formosana.* 2) The main branch of the tentacular nerve (Tn_1; Fig. 12A) arises at about midpoint on the tentacular nerve in *O. h. chiui* as it does in *Pomatiopsis lapidaria;* it arises from the base of the tentacular nerve in *O. h. formosana.*

(2) Lateral Aspect: The lateral complex involves the cerebral ganglion, pleuro-pedal and cerebro-pedal connectives. The right complex is shown in Figs. 11A, B. The lateral nerves are somewhat variable in position and number.

Lateral nerve 1 (Pp_1) is frequently not present (Fig. 11A vs 11B). Lateral nerve 2 (Pp_2) may be enlarged and later split into 2 at the lateral rostral wall. Lateral nerve 2 is the penial nerve in males. The origin of Pp_2 in *Oncomelania hupensis chiui* differs from that in *O. h. formosana,* where that nerve arises pressed against the origin of lateral nerve 3 (Pp_3). In *O. h. chiui* Pp_2 arises more dorsally from the pleuro-pedal connective (Pp). Lateral nerve 3 (Pp_3) arises and terminates the same way in both subspecies. Lateral nerve 4 described for *Oncomelania hupensis formosana* was not found in *O. h. chiui.*

In the left cerebral complex (Fig. 11D) Pp_1 is rarely present and when it is, it is very weakly developed.

(3) Cerebral Ganglion: The right cerebral ganglion is shown from exterior view in Fig. 11A (Cg). In Fig. 11C the medial surface is shown, to portray the exact position from which the cerebral nerves arise. The positions are the same as in *Oncomelania hupensis formosana.* Occasionally the cerebro-buccal nerve (Cb) is ventral to the sublabial nerve (Sul).

B. Pedal complex

(1) Anterior Aspect: The anterior aspects of the pedal, pro- and metapodial ganglia (Pg, Prg, Mg) are shown in Fig. 13. The greatest variability in the nervous system is found in the nerves (position and number) arising from or associated with the pro- and metapodial ganglia.

Consistent with *Oncomelania hupensis formosana* are the following:

(a) The elongate, cylindrical propodial connective (P_4).

(b) Nerves A and B arising, as shown, from the base of the pedal ganglion (Pg) or the beginning of the metapodial connective (P_6).

FIG. 13. Nervous system: pedal ganglion complex.

A. Anterior aspect of paired pedal ganglia and associated complex of nerves.

B. Varient in propodial connective showing an extra large lateral nerve (P_8) of the pedal ganglion.

C-F. Variation in nerves arising from right metapodial ganglion.

G-I. Variation in nerves arising from propodial ganglion.

J-M. Variations in the left metapodial complex.

A nerve from metapodial connective
B nerve from metapodial connective
Cp cerebro-pedal connective
Mg metapodial ganglion
P_1 lateral retractor nerve
P_2 nerve to antero-ventral wall of the pedal haemocoel
P_3 major lateral nerve of the pedal ganglion
P_4 propodial connective
P_6 metapodial connective
P_8 minor lateral nerve of the pedal ganglion
Pco pedal commissure
Pg pedal ganglion
Pp pleuro-pedal connective
Pp_3 lateral nerve 3 from pleuro-pedal connective
Prg propodial ganglion

TABLE 4. Comparison between forms of *Oncomelania hupensis* and *Pomatiopsis* of the sizes (in mm) of neural structures[4]

Neural structure	Dimension	Taxa		
		O. h. chiui mm	*O. h. formosana* mm	*P. lapidaria* mm
1. Cerebral ganglion (dorsal)	L	0.278–0.060	0.287*	0.290–0.360
	W	0.157–0.012	0.150*	0.238*
2. Cerebral commissure	L	0.073–0.097	0.070 ± 0.03	0.140–0.190
	W	0.061–0.097	0.06†	0.050–0.060
3. Total width of 2 cerebral ganglia and commissure	W	0.48–0.52	0.60*	0.76*
4. Pedal ganglion (anterior)	L	0.27†	0.24†	0.31†
	W	0.20†	0.22†	0.24†
5. Statocyst	D	0.09†	0.11†	0.12†
6. Buccal ganglion	L	0.14†	0.13†	0.19†
7. Pleural ganglion				
Right	L	0.154 ± 0.030	0.163 ± 0.300	0.240*
	W	0.105 ± 0.015	0.119†	0.120*
Left	L	0.121†	0.138†	0.170*
	W	0.121†	0.138†	0.170*
8. Pleuro-supraesophageal connective	L	0.287 ± 0.093	0.168 ± 0.050	0.34 ± 0.050
	W	0.054 ± 0.020	0.031†	0.041†
9. Supraesophageal ganglion	L	0.137 ± 0.016	0.100*	0.240*
	W	0.113 ± 0.014	0.080*	0.120*
10. Subesophageal ganglion	L	0.121†	0.138†	0.170†
	W	0.121†	0.138†	0.170†
11. Osphradio-mantle nerve (from "9" to the wall)	L	0.135 ± 0.030	0.412*	0.143*
12. Osphradial ganglion	L	0.385†	0.448*	0.571*
	W	0.125†	0.106*	0.142*
13. Visceral ganglion	L	0.159–0.227	0.283*	0.266*
	W	0.113†	0.133*	0.090–0.116

L = length
W = width
D = diameter
± = gives total variation

* = measurement from 1 individual
† = variability not encountered in measuring 6–7 individuals

[4] Measurements were taken from 6–12 adult individuals unless otherwise indicated. The total range is given without a mean value when only 6–7 measurements were made.

(c) The thin band-like metapodial connective (P_6) and often band-like metapodial ganglion (Mg).
(d) The pronounced major lateral nerve of the pedal ganglion (P_3).
(e) The irregular occurrence of P_8, the minor lateral nerve of the pedal ganglion.
(f) The position and strength of P_2, the nerve to the anteroventral wall of the pedal haemocoel.
(g) P_1, the lateral retractor nerve.

Nerves arising from the pro- and metapodial ganglia are very variable. Variation in the right metapodial ganglion and nerves is shown in Fig. 13, C-F; in the left metapodial ganglion, Fig. 13, J-M. Variation in the right propodial ganglion is shown in Fig. 13B, G-I.

(2) Lateral Aspect: The lateral aspect of the pedal ganglion, shown in Fig. 11A, corresponds with that of *Oncomelania hupensis formosana*. The lengths of the pedal ganglion and diameter of the statocyst in the 3 snails are compared in Table 4.

C. Buccal complex

The buccal ganglia are paired and connected to the cerebral ganglia by the cerebro-buccal connective (Cb; Fig. 11A).

Variation in nerves arising from the ganglion (Bg) are shown in Fig. 12, B-D.

The nerves are those found in *Oncomelania hupensis formosana*. The central buccal nerve (b3) is quite variable in position as shown in Fig. 12, B-D. The length of the right buccal ganglion is given in Table 4.

D. Pleural complex

The pleural ganglia (Lp, Rp) are shown in Fig. 11; 12A, E. These ganglia and associated nerves have the same shape and position as those in *Oncomelania hupensis formosana*. Their sizes are compared in Table 4.

The pleuro-supraesophageal connective (Psc; Figs. 11A, B, C; 12A) arises from the right plueral ganglion (Rp) and is noticeably longer and thicker than that found in *Oncomelania hupensis formosana*.

The most prominent nerve of the left pleural ganglion (Lp) is the mantel nerve (Mn_1; Figs. 11D; 12A, E) which runs postero-laterally to the cephalic wall where the mantel fuses with the wall.

The pleural ganglia are slightly smaller than those of *Oncomelania hupensis formosana* (Table 4).

E. Parietal complex

The parietal complex includes the supra- and subesophageal ganglia and the osphradial ganglion (Og; Fig. 10).

The supraesophageal ganglion (Sug; Fig. 12A) is more pronounced in *Oncomelania hupensis chiui* than in *O. h. formosana* (Table 4). From this ganglion arise 2 stout nerves or connectives; 1) the osphradio-mantle nerve (Omn; Fig. 12A) and 2) the supravisceral connective (Suv) to the visceral ganglion (Vg). The osphradio-mantle nerve runs 0.135 mm to the wall of the "neck," enters the wall as shown in Fig. 12A, and bifurcates, sending a mantel nerve to form a dialyneury with mantle nerve 1 (Mn_1) from the left pleural ganglion. The total length from supraesophageal ganglion to osphradium is 0.436 mm.

The relationship of subesophageal ganglion and left pleural ganglion is shown in Figs. 11D and 12A, E. The ganglia are either partially fused (Fig. 12E) or barely separated (Fig. 12A). The latter condition is more common and is what is normally found in *Oncomelania hupensis formosana*. As in *O. h. formosana*, 3 nerves, comparable in size and position, arise from the subesophageal ganglion: 1) mantel nerve 3 (Mn_3); 2) subvisceral connective (Sbv); 3) mid-columellar nerve (Mcn). The pronounced loop in the subvisceral connective (Sbv; Fig. 12E) is due to extreme contraction of the buccal mass. As shown in Fig. 12A, with protraction of the buccal mass the loop is pulled out. Comparison in length and width with *Oncomelania hupensis formosana* and *Pomatiopsis lapidaria* is given in Table 4. The ganglion is shorter and stouter than that of *O. h. formosana*.

F. Visceral complex

The position of the single visceral ganglion is shown in Fig. 5A. From the ventral aspect the ganglion (Vg) and 3 nerves are all that can be readily observed, i.e.,: 1) the subvisceral connective (Sbv), 2) the supravisceral connective (Suv), and, posteriorly, 3) the gonadal nerve (Gn).

With removal of some connective tissue and moving the position of the body and visceral ganglion slightly

TABLE 5. Comparison of radular size between 2 subspecies of *Oncomelania hupensis*

Feature	*O. h. chiui* (fr. 15 radulae)			*O. h. formosana* (fr. 16 radulae)			Significant difference
	$\bar{X}$	S	Se	$\bar{X}$	S	Se	
Radula: length (mm)	0.889	0.054	0.014	0.976	0.098	0.024	+ (P 0.01)
width	0.102	0.013	0.004*	0.120	0.008	0.002	+ (P 0.01)
Total number rows of teeth	77	4	1.03	84	7.5	1.9	+ (P 0.01)
No. rows of teeth in formative stage	15	4	1.03	19	5.3	1.4	None

*N = 11
$\bar{X}$ = mean
S = standard deviation
Se = standard error of the mean
+ = significant difference at 0.01 (1% level)

(Davis, 1967), one sees (Figs. 8; 12, F-I) that 3 nerves arise from the posterior ganglion and innervate various areas, as described for *Oncomelania hupensis formosana*. In addition to the gonadal nerve (Gn) there also arise the renal nerve (Rn) and external mantle cavity nerve 3 (E_3).

The pericardial nerve (Pn) varies in the position from which it originates from the supravisceral connective (Suv). As shown in Fig. 12, F-I, it most commonly arises about 0.11 mm from the visceral ganglion. A rare deviant is shown in Fig. 8, where the pericardial nerve arises from the visceral ganglion next to the root of the supravisceral connective.

The relationship of the visceral ganglion and associated nerves in males is shown in Fig. 9B. In both sexes the gonadal nerve travels posteriorly along the ventral side of the gonoduct.

Variations in the visceral ganglion and associated nerves are shown in Figs. 8; 12, F-I. Occasionally one finds fine nerve fibers passing to the mantel epithelium from the visceral ganglion (Var; Fig. 12G, H).

Exterior mantle cavity nerve 1 (E_1; Fig. 12F, G, I) corresponds to the same nerve in *Oncomelania hupensis formosana* in position and size. However, *O. h. chiui* lacks the strong kink in the subvisceral connective just posterior to the origin of E_1, found in *O. h. formosana*.

Exterior mantle cavity nerve 2 is not present in many specimens of *Oncomelania hupensis chiui*, though it is most often present in *O. h. formosana*.

The sizes of the visceral ganglia in the 3 taxa under comparison are given in Table 4.

9. Digestive System (Figs. 7; 11A, 14)

Three aspects of the digestive system are discussed in this section: 1) buccal mass, 2) stomach structure, 3) radula. Only the last is dealt with in detail.

The buccal mass has already been discussed in terms of external musculature above. It was thoroughly treated by Davis (1967) for *Oncomelania hupensis formosana*. The only aspect to be mentioned here is its length from rostral tip to cerebral commissure when the "mass" is fully contracted, which is slightly but distinctly smaller than that of *O. h. formosana* (see Table 3).

The ventral side of the stomach is

TABLE 6. A general formula for the most common cusp arrangement in *Oncomelania hupensis chiui* (from 30 radulae)

Tooth	General formula
Central ($\frac{\text{ant. cusps}}{\text{basal cusps}}$)	$\frac{2\text{-}1\text{-}2}{(3)2\text{-}2(3)}$
Lateral	2-1-3(4)
Inner marginal	7(8)
Outer marginal	6(7)

shown in Fig. 7, cleared of all overlying structures such as kidney tissue, reproductive organs (posterior section of prostate or pallial oviduct) and epithelium (Fig. 5A). The style sac (Sts), attached to the anterior chamber of the stomach (Ast), is conspicuous. The intestine (In) leaves the style sac as shown (Fig. 7) and discussed by Davis (1967). However, in that paper, the external morphology of the stomach is not so clearly shown.

The esophagus (Es) enters the stomach at the point of juncture of the posterior chamber (Pst) and anterior chamber (Ast). The single opening to the digestive gland is beneath the point where the vas deferens (Vd_1) crosses the boundary between digestive gland (Di) and stomach.

When the snails feed upon fine soil the fecal pellets (Fe) are rather uniform, solid, smooth and elliptical as shown in Fig. 7A. They are 0.40 ± 0.03 mm long and 0.18 ± 0.01 mm wide.

Radula (Fig. 14): Twenty radulae of laboratory bred snails were studied as well as 10 from field collected snails.

The length and width of the radula, the total number of rows of teeth, and the number of rows of teeth in the formative stage are compared with the same measurements for *Oncomelania hupensis formosana* (Table 5). The radula of *O. h. chiui* is distinctly smaller than that of *O. h. formosana*, i.e., it is shorter, more narrow, and has fewer rows of teeth.

TABLE 7. The various types of cusp arrangement for the different teeth in 30 radulae of *Oncomelania hupensis chiui* and the percentage of radulae showing that arrangement at least once

Central $\frac{\text{anterior cusps}}{\text{basal cusps}}$	%	Lateral	%
$\frac{2\text{-}1\text{-}2}{2\text{-}2}$	80	2-1-4 one side, 2-1-3 other side	50
$\frac{2\text{-}1\text{-}2}{3\text{-}3}$	70		
$\frac{2\text{-}1\text{-}1}{2\text{-}2}$	10	2-1-3	25
$\frac{2\text{-}1\text{-}2}{1\text{-}1}$	5	2-1-4	25
$\frac{2\text{-}1\text{-}1}{3\text{-}3}$	5	2-1-5	10
No. cusps inner marginal	**%**	**No. cusps outer marginal**	**%**
8	80	6	100
7	50	6 + 1	40
7 + 1	40	5	30
8 + 1	30	5 + 1	30
9	30	7	30
		7 + 1	20

+ 1 indicates the most lateral minute and often indistinct cusp.

In Table 6, the formula for the cusp arrangement most frequently encountered (i.e., occurring on 95% or more of the teeth on a radula or on different radulae) is given. The different types of cusp arrangements encountered at least once for each tooth are shown in Table 7.

The radula differs from *Oncomelania hupensis formosana* in that 1) the vast majority of centrals have 2 cusps on each side of the central anterior cusp while in *O. h. formosana* 62% of the radulae had

TABLE 8. Dimensional comparison for structures of the teeth (in μ) from field and laboratory reared *Oncomelania hupensis chiui*

Tooth	Feature	Laboratory (6 radulae)				Field (3 radulae)				Level Sign. diff.
		No.	$\bar{X}$	S	Se	No.	$\bar{X}$	S	Se	(P)
Central	A	12	25.2	2.11	0.61	7	26.3	2.42	0.91	-
	B	21	11.9	1.13	0.26	9	14.6	2.18	0.73	+ (.01)
	C	21	12.6	0.95	0.21	9	15.6	1.02	0.34	+ (.01)
	D	21	10.2	0.21	0.05	9	12.7	3.77	1.26	-
Lateral	L	16	45.8	1.44	0.36	6	47.3	2.09	0.85	-
	W	20	17.5	1.26	0.28	9	18.5	0.29	0.10	+ (.01)
Inner Marginal	L	21	40.9	2.84	0.62	8	45.4	2.63	0.93	+ (.01)
	W	30	16.5	1.08	0.20	7	15.7	0.75	0.28	+ (<.05)
Outer Marginal	L	21	42.9	2.70	0.59	5	46.6	3.20	1.42	+ (.02)
	W	14	13.7	0.96	0.25	7	14.6	0.98	0.37	-

A = width of base (posterior edge) of central tooth
B = anterior width
C = distance between tips of 1st basal cusps
D = distance from anterior edge of the tooth to the tip of the basal cusps
L = Length
W = Width

$\bar{X}$ = mean
S = standard deviation
No. = number of teeth measured
Se = standard error of the mean
P = probability level
- = not significantly different
+ = significant difference

but one in at least 90% of the individual teeth. 2) Many inner marginals of *O. h. chiui* have 7 cusps (50% of the radulae have this count at least once) while 8 or more are common in *O. h. formosana*. 3) In *O. h. chiui* the outer marginal has 7, or 7+1, cusps in 50% of the radulae at least once, while only 4-6 are found in *O. h. formosana*. Referring to 7+1, the +1 indicates the most lateral cusp which is often very minute and not distinct when the tooth is observed from certain positions. It is most evident when the tooth is bent back to expose clearly all cusps in one plane. 4) A cusp arrangement of 2-1-4 in the lateral teeth is common in *O. h. chiui* (at least once in 75% of radulae) while rare in *O. h. formosana* (10%).

While there is distinct overlap in cusp number, there are marked differences in frequency of cusp number as shown above. Of interest is the fact that in 50% of the radulae the lateral tooth had 2-1-3 cusps on one side of the central while having 2-1-4 on the other side. This arrangement extended the whole length (80%) or more of the rows where the formula could be discerned) of the radula. In *O. h. formosana* this asymmetry occurred in 10% of the radulae.

In studying the radulae of field snails, 2 distinct differences between these and laboratory reared snails were found: 1) in the size of portions of the central tooth, 2) in the cusp formula of the central tooth.

The dimensions for the teeth of the laboratory and field snails are given in Table 8. There was a significant difference in the 2 measurements of the central tooth: 1) width of the anterior edge of the tooth, 2) distance between the tips of the basal cusps (Bc; central 1, Fig. 14), which was greater in the field snails. Also, the width of the lateral tooth, as well as the lengths of the inner

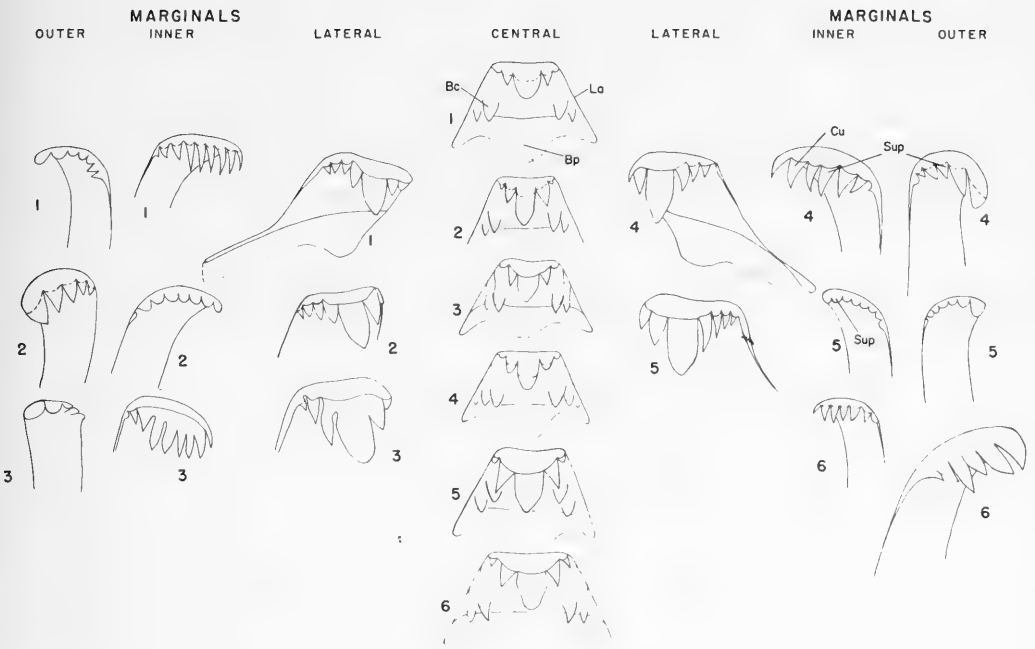


FIG. 14. A few teeth of each type found on the taenioglossate radula of *Oncomelania hupensis chiui* are given to demonstrate variation in size, shape and structure. Each cusp of a tooth is composed of a support (Sup) and dagger-like cutting edge (Cu). The supports are thickened; depending on the plane of focus, they may not be distinct (outer marginal 6) in which case the ends of the supports are represented by broken lines (e.g., outer marginals 2, 4, etc.). At another level of focus they are evident (e.g., inner marginal 4, laterals 1, 4, etc.).

The cutting edges of several cusps may grow out beyond the basal supports and fuse to form a bizzare enlarged cutting edge, e.g., inner marginal 3, lateral 3.

In preparing the radula for study, cutting edges may have been accidentally broken away from the supports, e.g., in outer marginals 1, 3, 5, etc.

Bc	basal cusp	Cu	cutting edge of the cusps
Bp	basal process attaching central to lingual membrane	La	lateral angle of the central tooth
		Sup	support for cutting edge

and outer marginals, in the field snails were significantly greater (P at the .01 level) whereas the width of the inner marginal of the field snails was significantly less than that of the laboratory snails ($P < .05$).

In 1 or 2 field snails a central cusp formula of 2-1-2/4-4 was found. The 4th basal cusp on each side was tiny but distinct.

Occasionally one of the marginals was larger than usual in the field snails (outer marginal 6, Fig. 14).

The teeth shown in Fig. 14 are presented to show variation. In some instances the cutting edge (Cu) of the more pronounced cusps of the lateral or inner marginals grew considerably beyond the basal supports (Sup) to form a wide, fused cutting edge with pronounced

jagged denticles (lateral 3, inner marginal 3 contrasting with the more normal lateral 1, inner marginal 1).

10. Conclusion

On the basis of this anatomical study and detailed comparison with *Oncomelania hupensis formosana*, it is evident that the so-called *"Tricula chiui"* is, indeed, a member of the *Oncomelania hupensis* complex. With a few minor exceptions, qualitative aspects of anatomy are the same. The exceptions are: 1) lack of nerve Pp_4 (lateral nerve 4 from the cerebro-pleural complex) present in *O. h. formosana* but not demonstrated in *O. h. chiui*. This nerve was not always demonstrated in *Pomatiopsis lapidaria* or *O. h. formosana* and when it was not found it was "suspected that it was incorporated within the pleuro-pedal commissure" (Davis, 1967). 2) The origin of nerve Tn1 from the midtentacular nerve instead of the base of the nerve. 3) The number and arrangement of medio-lateral slips of the buccal protractor (M_5) as discussed in the section on the muscular system (p 32). Details of female and male anatomy are identical in the 2 snails. Differences in structure compared with other genera (mainly *Pomatiopsis*) are those discussed by Davis (1967).

Most differences from *Oncomelania hupensis formosana* are connected with the smaller size of *O. h. chiui*, i.e., the organs are generally smaller. All such differences are thus considered a correlate of 1 factor, smaller size.

Nevertheless there exist several differences important enough to assign the taxon to subspecific rank apart from *Oncomelania hupensis formosana*.

1. Although the length of gill in the 2 subspecies is the same, *O. h. chiui* has distinctly fewer gill lamellae (under 36 as against over 42).

2. The pleuro-supraesophageal connective of *O. h. chiui* is distinctly and significantly longer.

3. The supraesophageal ganglion is distinctly of greater size in *O. h. chiui*.

4. There are differences in the frequency with which different cusp numbers occur on the various teeth on the radula, that are thoroughly discussed in the section on the radula.

5. The shell has potential for forming only a very vague varix.

These 5 major anatomical differences together with the uniformly smaller size would be taken by many to justify full specific status. The point will be further discussed below when hybridization, electrophoretic and serological data are considered.

TABLE 9. Cultures involving the mating of *Oncomelania hupensis chiui* with *O. h. formosana* and *O. h. quadrasi* and their productivity

Subspecies of *Oncomelania hupensis*			Duration[5] (months)	Young/ female/ day
chiui		*formosana*		
5 ♀	x	5 ♂ (Yueh Mei)	9	0.12
5 ♀	x	5 ♂ (I-lan area)	9	0.05
5 ♂	x	5 ♀ (Pu Yen)	7	0.22
5 ♂	x	5 ♀ (Pu Yen)	7	0.26
5 ♂	x	5 ♀ (Yueh Mei)	7	0.29
5 ♂	x	5 ♀ (Yueh Mei)	7	0.12
chiui		*quadrasi*	5	
2 ♀	x	5 ♂		0.00

[5] The cultures were started in January, March and May, and were all terminated in October, 1965.

HYBRIDIZATION STUDIES

1. Materials and Methods

Five males or 5 (2) females of *Oncomelania hupensis chiui*, resp. of *O. h. formosana* of various strains and also 5 male *O. h. quadrasi* were placed in cross cul-

ture as shown in Table 9. Males were uniformly 3-4 months old. Females were reared singly in Petri dishes as described by van der Schalie & Davis (1965) from the 2.5 whorl stage. Females were maintained in isolation until an age of 2.5 months and were then placed in culture.

The breeding vivarium was the medium clay pot, kept at room level light, as described by Davis (1967) and van der Schalie & Davis (1968). Cultures were checked monthly for young. Each month the sex of all snails was checked in all cultures to ascertain whether a male of the wrong subspecies had been erroneously included. All young were removed each month.

2. Results

As shown in Table 9, all cultures produced young, except the cross involving *Oncomelania hupensis quadrasi*. The rate of production varied from 0.05-0.29 young per female per day over the entire period of 7-9 months, when the crossmatings involved *O. h. formosana*.

3. Discussion

This series of crosses provides no more than initial data on the potential of *Oncomelania hupensis chiui* to form hybrids with the other subspecies of *Oncomelania hupensis*. The negative results obtained with *O. h. quadrasi* are not reliable in view of the low number of females and the single culture involved.

The rate of reproduction was at a level corresponding to that for *O. h. formosana* reared under identical conditions. The latter produced 0.17-0.33 y/f/day in their first year in culture (van der Schalie & Davis, 1968).

These initial results substantiate that *Oncomelania hupensis chiui* is, indeed, closely related to *O. h. formosana*. Further crossing studies should be done to assess relationships to the other subspecies of *Oncomelania hupensis*.

ELECTROPHORETIC ANALYSIS

1. Introduction

The value of electrophoresis is covered in terms of data and literature survey in the reference volume Taxonomic Biochemistry and Serology (1964). Cheng (1964) reviewed some previous work pertaining to electrophoreses and molluscan systematics; Davis & Lindsay (1967) provided other references. Additional papers are those of Targett (1963), Wright & Ross (1966) and Wright, File & Ross, (1966).

The present study involves comparing the electrophoretic "fingerprint" patterns of *Oncomelania hupensis chiui* with those of 3 field collected populations of *O. h. formosana:* 2 from I-lan county (church and airport populations) and 1 from the Pu Yen village area, Changhua county (Fig. 1). In addition, laboratory reared F_1 and F_2 generations of I-lan parental stock (church population) were studied.

The advantages and disadvantages of using polyacrylamide electrophoresis for this type of study are discussed by Davis & Lindsay (1967). As stated by them this method has the advantages of being sensitive enough to demonstrate variation between populations of a species when such variation occurs. High resolution of numerous protein fractions (15-26) is obtained in a sample with as little as 200-300 micrograms of protein.

2. Materials and Methods

The source of proteins was foot muscle extract. Foot tissue from 20-50 snails was pooled for homogenization as discussed by Davis & Lindsay (1964, 1967) and Davis (1967). Justification for pooling tissue was discussed by them.

Polyacrylamide (=disc) electrophoresis was employed. The standard 7.5% acrylamide gel and tris-glycine buffer (pH 8.2-8.4) were routinely used as discussed by Davis & Lindsay (1967). From 3-10 experiments were made for each population. Each experiment comprized 5-10 gel tubes. Human blood serum was used in controls (see below) for determining whether the gels of each experiment were optimal, good or poor. Gels were stained in amidoschwartz for 2 hours and destained electrically in 7.5% acetic acid. Stained gels (uniformly 34-35 mm long) were analyzed for densi-

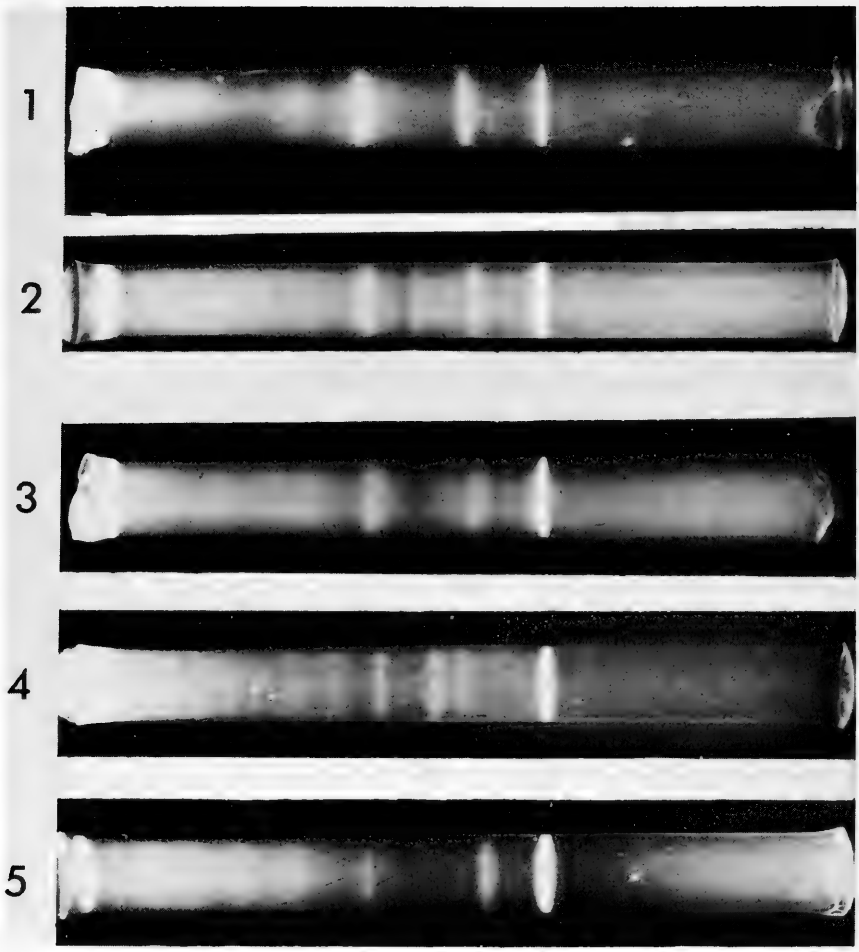


FIG. 15. Disc-gel tubes with characteristic electrophoretic separation patterns for foot tissue proteins from the 5 *Oncomelania* populations studies. The stained gels (34–35 mm long) were placed directly under an enlarger and the distribution pattern was printed, with the gel column serving as a negative.

1. *Oncomelania hupensis chiui.*
2. *O. h. formosana,* I-lan church population.
3. *O. h. formosana,* I-lan airport population.
4. *O. h. formosana,* Pu Yen population.
5. *O. h. formosana,* I-lan laboratory population.

tometric patterns by means of a Canalco Model E microdensitometer.

Data are discussed in terms of optical densitometric pattern and of Rf values (ratio of distance from the origin to a given fraction to distance from origin to the front). The former is the result of component position and density while the latter indicate only component position. Methods used for determining Rf values

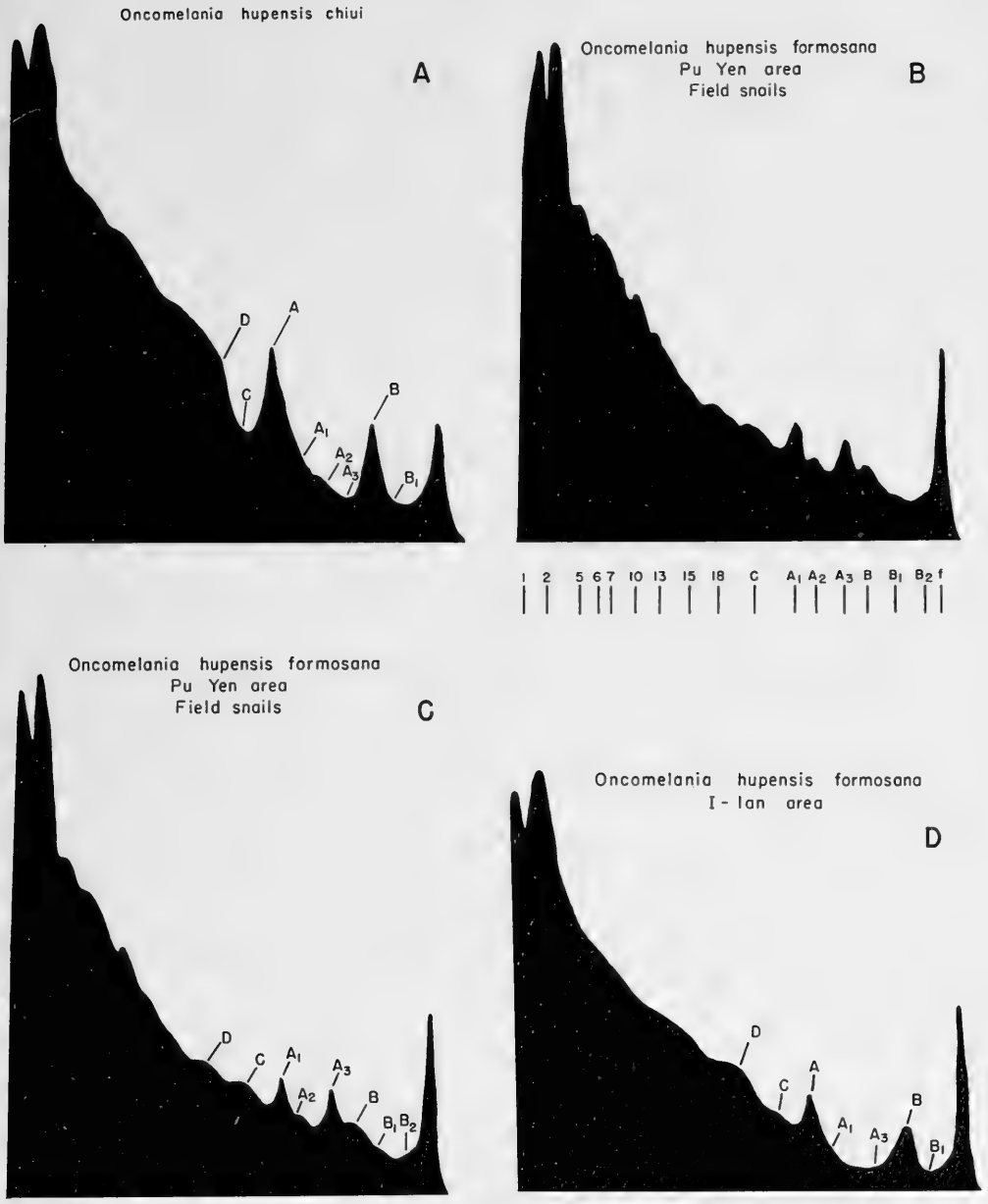


FIG. 16. Densitometric tracings of the electrophoretic protein distribution patterns of 3 *Oncomelania* populations. Letters marked in Figs. A, C, D (and right half of B) are used in the text to discuss profile patterns as well as component position in the frontal, most characteristic part of the profile (gel area 2). In Fig. B the individual components in the initial part of the profile (gel area 1) are numbered. The profile given in C is of a different run than the one shown in B; it serves to demonstrate reproducibility of profiles from different experiments and aids in comparing the different types of densitometric pattern found in A and D. Fig. D is a generalized pattern for the I-lan field snails.

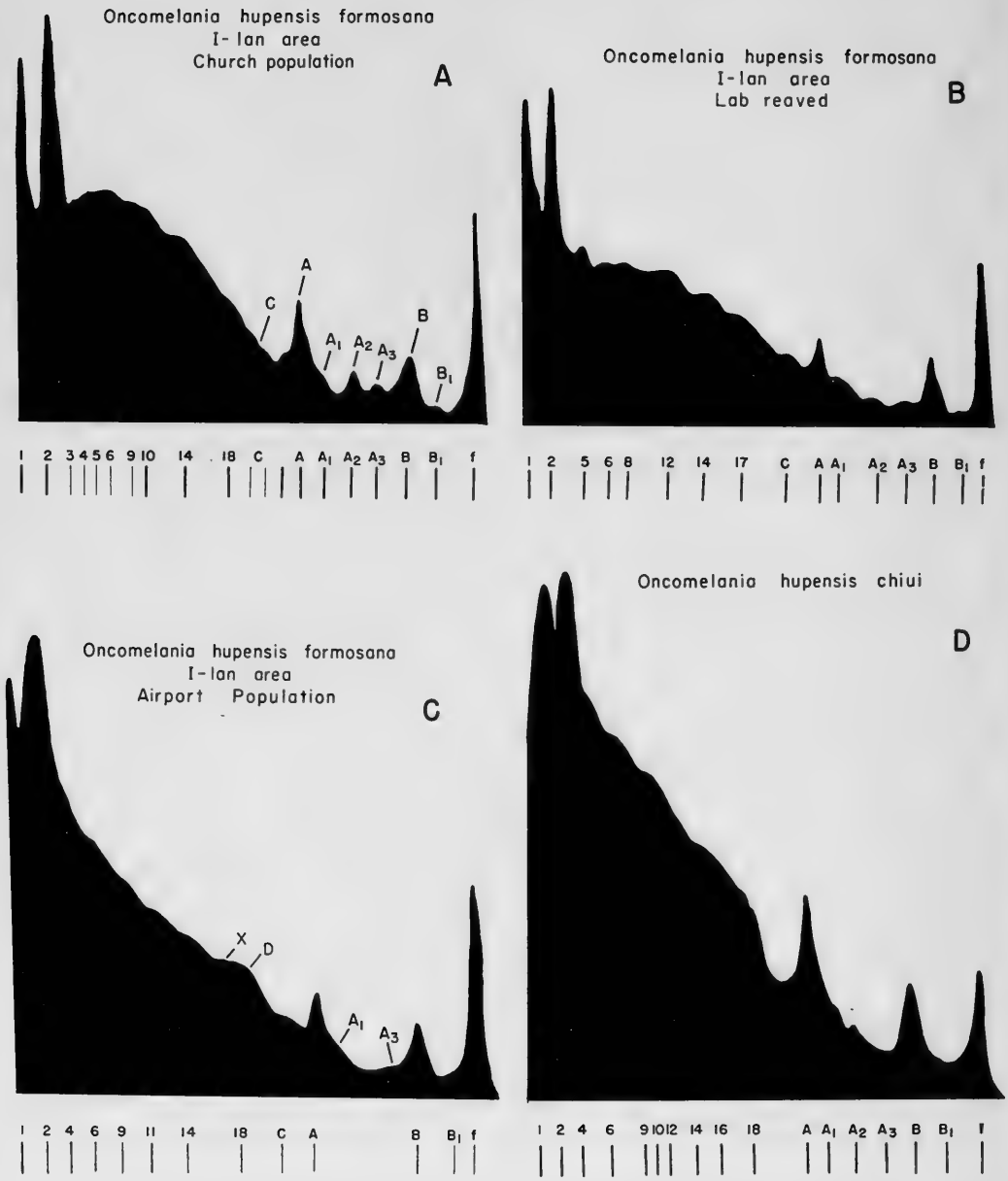


FIG. 17. Densitometric tracings of the electrophoretic patterns of 4 *Oncomelania* populations. The position of each protein component is marked by numbers in gel area 1, and by letters in gel area 2. The same symbol, in the different populations, indicates the same Rf value for the components thus represented.

TABLE 10. Average Rf values for components in gel area 1[6], for the 5 populations of *Oncomelania hupensis* studied

Component No. in gel area 1	*O. h. chiui*	*O. h. formosana*			
		I-lan			Pu Yen
		Church	Airport	Lab	
1	.015	.014	.014	.014	.014
2	.064	.066	.066	.065	.071
3	*	*	*	*	*
4	.117	.115	.115	*	-
5	*	.138	*	.138	.133
6	.174	.188	.179	.176	.183
7	-	-	-	-	.211
8	-	-	-	.223	-
9	.247	.244	.230	-	-
10	.276	.271	-	-	.266
11	-	-	-	-	-
12	.303	.290	.288	.291	-
13	-	-	-	-	.326
14	.373	.377	.368	.377	-
15	-	-	-	-	.392
16	.247	-	-	-	-
17	-	-	-	.456	-
18	.477	.478	.472	-	.465
No. of component differences compared to *O. h. chiui:*		1	2	5	7

* = fractions resolved only under optimal gel conditions.

[6] Area of dense, slowly migrating, large molecular weight proteins (see Figs. 16, 17). The average valves were derived from 8-10 tubes from 3-10 different experiments.

are discussed by Davis & Lindsay (1967).

Rf values were determined for each fraction in all tubes and are presented as averages. As separations of proteins from homologous preparations yield stable patterns, the standard deviation for an average Rf value rarely exceeded the error arising when 2 persons measured the same component, i.e., 3%, or an Rf value of 0.014. Total variation in a long series of measurements from a number of homologous runs rarely exceeded 0.045 and more commonly was 0.020 or less. It was determined that, in general, when average Rf values differed by 0.018, they were very significantly different statistically. When Rf values are 0.017 apart, it is necessary to determine by calculation whether or not they are significantly different.

3. Results

The typical electrophoretic patterns for each of the 5 populations investigated, i.e., the stained gel columns themselves, are shown in Fig. 15. For ease in comparing the densitometric profiles (Figs. 16, 17) drawn from typical electrophoretic separations, the linear sequence of components was divided into 2 areas. Area 1 extends from the origin (fraction 1) to point D (resp. fraction 18). In this stretch of gel are numerous closely

TABLE 11. Average Rf values for components in gel area 2[7] for the 5 populations of *Oncomelania hupensis* studied

Component in gel area 2	*O. h. chiui*	*O. h. formosana*			
		I-lan			Pu Yen
		Church	Airport	Lab	
C	.544	.550	.546	.557	.555
A	.601	.614	.632*	.624*	-
A_1	.652	.657	-	.674*	.645
A_2	.721	.729	-	.751**	.705
A_3	.780	.786	-	.823**	.762*
B	.831	.857*	.859*	.881**	.821
B_1	.896	.914	.923*	.940**	.888
B_2	-	-	-	-	.969*
Front	1.00	1.00	1.00	1.00	1.00
No. of component differences compared to *O. h. chiui:*		1	6	6	3

* = Rf significantly different from *O. h. chiui*

** = Rf significantly different from both *O. h. chiui* and the church population

[7] Area of more motile proteins with lower molecular weight (see Figs. 16, 17). The average values were derived from 8-10 tubes from 3-10 different experiments.

packed protein fractions of relatively high molecular weight; they are dense and slowly moving components. This region is characterized by a tendency for a dark gel background due to diffuse protein complexes.

Area 2 starts at C (Figs. 16, 17) and continues to the front (f). It contains rapidly migrating proteins which are usually not extremely dense. In this second area fractions tend to be more widely spaced and distinct. Area 2 is characterized by relative stability in fraction density and resolution under gel conditions causing distortion, diffusion of components, or difficulty in resolving components in area 1.

Variance in sample concentration, gel conditions, and length of electrophoretic separation have an effect on the number and resolution of components in area 1. In human blood controls optimal gel conditions resulted in resolving over 22 fractions and resolution of post albumen fractions (bands between transferrin* and albumen) was excellent. Less than optimal conditions resulted in resolving only 19-20 fractions while post albumens were fuzzy or 1-2 were absent. When 19-20 fractions of human serum proteins were resolved and all post albumens were observed, gel conditions are considered good. Most results presented here were obtained when gel conditions were good or optimal. With optimal conditions several more bands were resolved in area 1 (if run lengths were the same). For instance, component X (Fig. 17C) could, at times, be resolved from the relatively wide, diffuse fraction resulting in densitometric peak D.

The average Rf values for components in the initial, dense region (gel area 1)

* Transferrin is the iron binding protein in serum that is very prominent in polyacrylamide electrophoresis when serum components are being separated. The component was shown by Ornstein (1962) in relationship with other human serum components.

for the populations studied are presented in Table 10. The following differences in the congruency of components were noted between *O. h. chiui* and the other populations: 1 difference from the I-lan church population, 2 and 5 differences from I-lan airport and laboratory populations respectively; there were 7 differences from the Pu Yen field population.

Certain bands (marked with an asterisk in Table 10) are resolved only when gel conditions and protein concentrations are optimal. In all populations proteins between Rf values of 0.392 and 0.477 may appear blurred, with denser areas representing band centers. These fractions are only clearly resolved under optimal gel conditions and even then there is a stained, blurred area between the components.

In the frontal gel area 2 there are 2 different types of densitometric pattern found in the 5 populations. Type I includes *O. h. chiui* and all populations of I-lan *O. h. formosana* (Figs. 16A, D; 17A-D). Type II corresponds to the Pu Yen population of *O. h. formosana* (Figs. 16B, C).

In the former pattern (Type I) the 2 fractions at positions A and B are widely separated, dense components. Between A and B are 3 minor fractions: A_1 - A_3. In the latter pattern (Type II), 3 distinct, dense components are observed, but they correspond to positions A_1, A_3 and B. The density of the fraction at B is regularly quite less than that of the fractions A_1 and A_3 (Fig. 16B, C).

The fraction at position C in Type I is not dense, and in *O. h. chiui* it is frequently not resolved. The result in densitometric pattern due to this faint fraction is a marked dip between position A and D (D represents the end of gel area 1) (Fig. 16A). As shown in Table 12, fraction C was not resolved in more than 75% of the tubes of experiments yielding a Type I densitometric pattern. In the Type II pattern (Pu Yen population) the fraction at position C was present in 100% of the tubes of all experiments and was characteristically quite dense (Fig. 16B, C), often appearing as 2 dense, slightly separated bands.

TABLE 12. The percentage of tubes of all experiments in which component C was resolved for the 5 populations of *Oncomelania hupensis* studied

Snail populations studied	%
O. h. chiui	57
O. h. formosana	
I-lan populations:	
Church	75
Airport	75
Laboratory	69
Pu Yen population	100

Considering the Type I pattern, fractions at positions A_1 - A_3 were distinct for *O. h. chiui* but at a very low density. Component A_1 had a tendency not to resolve into a distinct band and often appeared as a diffusely stained area closely associated with A. A_2 was never resolved in the airport population of *O. h. formosana* (Fig. 17C) while A_1 and A_3 were either faint diffuse bands or unresolved. A_1, A_2 and A_3 were all distinct and of moderate density in the church population (Fig. 17A).

Component positions A, B, C, etc. were used in discussing reference points in the 2nd area of the densitometric tracings or patterns. The actual Rf values for the fractions pertaining to these patterns are given in Table 11. The average Rf values of the various populations of *O. h. formosana* are compared with those of *O. h. chiui* and the number of corresponding fractions is listed. It is seen that Rf values corresponding to the same peak in the densitometric tracings can significantly differ. At position B, for example, *O. h. chiui* significantly differs from all I-lan snails, while the laboratory population, descended from the church I-lan snails, significantly differed from both the field snails and *O. h. chiui*.

On the basis of Rf values alone, in the 2nd gel area, *Oncomelania hupensis chiui* most closely corresponds to the I-lan church population (1 difference) and least of all, to the laboratory and airport populations (6 differences). The low correspondence of fraction position between populations of I-lan snails will be discussed later. The Pu Yen snails are separated from all the other populations on the basis of a different densitometric profile, i.e., different fraction position and density.

Oncomelania hupensis chiui has distinct components at Rf 0.117, 0.174 and 0.247. Rarely is a faint band resolved at 0.138; in this respect it differs from the church and laboratory I-lan populations of *O. h. formosana* where Rf 0.138 is very prominent.

4. Conclusion and Discussion

The results indicate that electrophoretically, *Oncomelania hupensis chiui* is nearly identical with the church population of I-lan *O. h. formosana.*

Basically, the electrophoretic patterns of all populations studied are quite similar. As previously mentioned (Davis, 1967), all the subspecies of *Oncomelania* are characterized by at least 1 dense, distinct fraction at an Rf beyond 0.750. In the American *Pomatiopsis lapidaria*, a hydrobiid snail most closely related to *Oncomelania*, there are no dense fractions beyond 0.750, and, although there are other distinctive features, this difference is the most outstanding.

As already stated, the Pu Yen population of *Oncomelania hupensis formosana* differs from the others, in gel area 2, in pattern, i.e., both in density of fraction and Rf. These differences, coupled with the many (7) differences in Rf in area 1, sets the Pu Yen population aside from the others.

Concerning the I-lan field populations of *O. h. formosana*, the airport population appears different because of the lack of distinct A_1 - A_3 fractions in gel area 2. As mentioned above, A_1 and A_3 are present as diffuse bands, while A_2 was not resolved. In the laboratory population the whole pattern of C to B_1 has shifted towards the front, relative to the church population, so that the Rf values appear different. The pattern itself, however, remained the same, indicating homology of fractions despite a global shift. In the I-lan populations of *O. h. formosana* (laboratory and field) and in *O. h. chiui*, there is a tendency for increased resolution of A_1 - A_3 as follows: from poor in the airport population, and slightly more pronounced in the laboratory population and *O. h. chiui* to very pronounced in the church population.

The laboratory population appears different from the field populations because of the above mentioned global shift of fractions. It should be further tested to what extent laboratory bred populations, always descended from a narrower selection, differ from field snails. One would conjecture that changes in electrophoretic patterns would range from none, or very slight, to a degree indicating a population difference as marked as that separating the Pu Yen field snails from the I-lan snails. It is doubted, however, that a difference tantamount to a species difference would occur.

The electrophoretic differences observed between these populations are reproducible and characteristic. A discrete difference indicates a taxon difference. Assigning such differences to categories of species, subspecies, or population variants, depends on 2 groups of facts: 1) On how electrophoretic data fit in with those involving other characters; 2) On the magnitude of electrophoretic difference found between taxa which are accepted as distinct on many criteria, yet clearly belong within the same genus.

The assignment of these populations to categories is reserved for the final discussion, where electrophoretic considerations will be a factor among others in assessment.

IMMUNOLOGICAL STUDIES

1. Introduction

Immunological studies were undertaken to determine the relationship between *Oncomelania hupensis formosana* and *O. h. chiui* in terms of homogeneity or heterogeneity of antigens. The comparison also involved 3 other subspecies of *O. hupensis*.

Relatively few papers deal with immunological studies on mollusca that involve systematic relationships or taxonomy. Pertinent references are those of Morrill, Norris & Smith (1964), Michelson (1966a, b) and Wright & Klein (1966).

2. Materials and Methods

a. Antigen preparation and production of antisera

The source of antigens was foot muscle extract of *Oncomelania hupensis formosana* from Pu Yen, prepared exactly the same way as if it were to be used for electrophoresis (Davis & Lindsay, 1967). Only freshly prepared extracts were used in all experiments and to produce antisera. The extract in the immunodiffusion experiments was prepared as follows: 0.30 gm of blotted wet weight of tissue was homogenized in 2.0 ml Carriker's Saline as described by Davis & Lindsay (1967), the homogenate was centrifuged at 250 xg for 5 minutes and the supernatant (= extract) decanted. All operations were carried out at 2-5° C. The protein content of the extract was determined using the Biuret reaction (fide Kabat & Mayer, 1961) and a standard curve was established using purified crystalline bovine albumen (clinical pathology standard). Readings from the Biuret reaction were made with a Shimadzu QR-50 spectrophotometer. Snail foot extract, as prepared above, yielded 6.0-7.0 mg/ml of protein.

White rabbits (5-6 lbs; virgin female) were used to produce antisera. Foot tissue extract was prepared at the concentrations indicated in Table 13, column 2. The stated weight of tissue was homogenized per 1 ml saline and treated as described above. The extract thus obtained was injected into the rabbit intravenously via a lateral ear vein, in 2 injection series, 2 months apart (see Table 13 for schedule). A total of 7-8 mg of protein was injected in each series. Rabbits were bled from the ear 5 and 10 days after the last injection in the second series. It had been previously established that the strongest and best defined antigen-antibody precipitin systems resulted from serum obtained 5 days after the last injection, and that these were somewhat weaker with 10th day serum.

Antiserum was pressed through a sterile millipore filter (0.45μ) into presterilized tubes. Merthiolate (aqueous 1:1000) was added in the proportion of 1:10 as a preservative and the serum was stored at 3° C for the duration of the experiments.

Although 4 or 5 rabbits were used to produce antiserum, the 5th, resp. 10th day antiserum from only 1 of these was used in all experiments here reported, because tests had revealed their greater specificity and higher quality, as evidenced by the number and strength of precipitin systems.

b. Diffusion Techniques

Two tests were utilized in this study. (1) Micro-Ouchterlony double diffusion procedures were carried out; these made it possible to test through in-gel specific absorption of antiserum by antigens (and consequent precipitation), the homogeneity or heterogeneity of relevant antigens in the foot muscle extracts of a population, with regard to the corresponding antigens from Pu Yen snails. (2) Immunoelectrophoresis was used to demonstrate the position of separated relevant antigens in the acrylamide gel columns, so as to permit a comparison of the protein bands making up a taxon-specific densitometric profile (discussed in the electrophoretic section) and the

TABLE 13. Schedule for intravenous injections of snail[8] foot muscle extract into rabbits for antibody production

Time schedule (Days)	Foot tissue homogenized mg blotted wet weight per 1.0 ml saline	mg protein injected	
		$\bar{X}$	Se
1	5.55	0.60	0.143
3	13.69	0.78	0.145
5	27.75	1.06	0.066
7	55.50	2.46	0.156
9	83.25	2.75	0.000
2 month interval		7.65 total	
1	5.55	0.60	0.143
3	13.69	0.78	0.145
5	27.75	1.06	0.066
7	55.50	2.46	0.156
9	83.25	2.75	0.000
14 (=5th day) bled from 19 (= 10th day) the ear		7.65 total grand total 15.30	

$\bar{X}$ = mean value
Se = standard error of the mean

[8] *Oncomelania hupensis formosana*, Pu Yen population

position of antigens which were involved in the double diffusion tests (it will be remembered that snail foot extract was prepared in the same way for both electrophoresis and immunological specific absorption tests).

Agar, used in both types of tests, was prepared as follows: Non-nutrient agar (Difco Company, Detroit, Michigan, U. S. A.) was made up to 2% in distilled water, cut into blocks, and rinsed in cold water for 12 hours. The agar was then stored in distilled water in the refrigerator for several days, changing the distilled water daily, for removal of soluble impurities. In preparation for the tests the 2% agar was dissolved in 0.90% saline to form a 0.45% final saline concentration, merthiolate was added as a preservative to a final concentration of 1:10,000 and the final concentration of the agar was 1%.

In all tests, extracts and antisera were used undiluted. When the tests were initiated by permitting double diffusion (i.e., of antigens and antibodies) to commence, the experiment proceeded in a moist environment for 3 days at 21° ± 1°C (3-5 days for immunoelectrophoresis) to allow for proper diffusion and precipitation. Resulting opaque precipitin patterns were recorded by placing unstained gels under a photographic enlarger and using them as negatives. The arc patterns were thus printed out on photographic paper and are reproduced in Figures 18-25. Tracings were also made of the precipitin arcs so as to safeguard against loss of detail in final reproduction, especially as several systems were light and closely associated.

Control serum did not react with saline or extract.

1) Specific absorption tests (based on double diffusion principles and using micro-Ouchterlony techniques). Tests were conducted in agar contained in

rings (inside diameter 22 mm), cemented to microscope slides (75 x 25 mm) with a paraffin-wax mixture. Agar (1%) at 60°C was poured into the rings (2.5 ml/ring) and allowed to solidify. Four wells arranged in a diamond pattern were cut in the agar (Figs. 19-25) using a template designed by Dr. George Nace, University of Michigan, Ann Arbor. The centers of the wells on the long axis were 16 mm apart, those on the short axis 12 mm apart. Each well had a diameter of 4 mm and a capacity of 82-85 µl.

The immunodiffusion tests were conducted as explained in the following example, where 2 populations of *Oncomelania hupensis formosana*, one from Pu Yen and one from Yueh Mei, and anti-Pu Yen serum were used (Fig. 19B). Foot tissue extract of snails from Yueh Mei (heterologous extract, since the homologous systems involve extract of Pu Yen snails and anti-Pu Yen serum) was placed in well labeled Abs-YM. At that time no other wells were filled. The extract was allowed to diffuse through the gel for 12 hours at which time components had migrated to a distance of 1.5-2.0 mm from the well. At 12 hours the excess extract was removed from the well, thus slowing considerably the rate of diffusion of the extract. Then, fresh heterologous extract from Yueh Mei snails was placed into well YM and fresh homologous Pu Yen snail extract was placed into well PY while anti-Pu Yen serum was placed into the well marked Abs-YM and into the unmarked well.

The antiserum in the Abs-YM well then diffused through the barrier of previously diffused heterologous Yueh Mei antigens around the periphery of the well with ensuing absorption of relevant antibodies. The absence of precipitin bands between wells Abs-YM and YM showed that absorption was complete, i.e., that all relevant antigens of the Yueh Mei extract that could react with anti-Pu Yen serum had precipitated out around well Abs-YM. The presence of bands between the Abs-YM and PY wells (compare Figs. 23-25) would indicate antigens unique in, or partially particular to, the Pu Yen snails. The absence of such bands in Fig. 19B indicates that the antigens of the 2 populations here investigated were homologous. In contrast, precipitin arcs did result from the interaction of the non-absorbed antiserum diffusing from the unmarked well (bottom), which had not previously contained antigen, and the antigens diffusing from the (lateral) wells marked YM and PY. The number of arcs which formed around the unmarked well indicate the number of antigens harboured in common by the 2 populations and reacting with anti-Pu Yen serum.

2) Immunoelectrophoresis. Unstained polyacrylamide gels (i.e., gel columns comprizing the "native" components electrophoretically separated in the "lower" gel (to the right of the spacer gel (S.g.) in Fig. 18) as well as those still present in the sample (Sa.g.) and spacer (S.g.) gels were laid along one side of glass slides (1 gel column per slide) and cooled agar was poured onto each slide around the electrophoresed gel. Upon solidification of the agar, 2 thin strips of filter paper were soaked with antiserum and were placed, one atop the other, on the agar, parallel to and 5 mm away from the gel column. At the end of 3-5 days the filter paper strips were removed and the precipitin arcs which had formed were recorded as described above. In Fig. 18, the filter paper strips have been removed and the 2 slides placed side-by-side. The sample gel (Sa.g.) was lost from the upper gel column after the reactions were completed. The dark strips over the gels to the right of the spacer gels (S.g.) are artifacts.

Foot muscle extracts used in the immunoelectrophoretic tests were from Pu Yen *O. h. formosana* and *O. h. nosophora*. The antiserum was the same (5th day anti-Pu Yen serum) as that used in the specific absorption tests.

3. Results

As shown in Fig. 18, the sources of antigens operative in specific absorption tests were fractions of a slowly migrating nature of high molecular weight. The

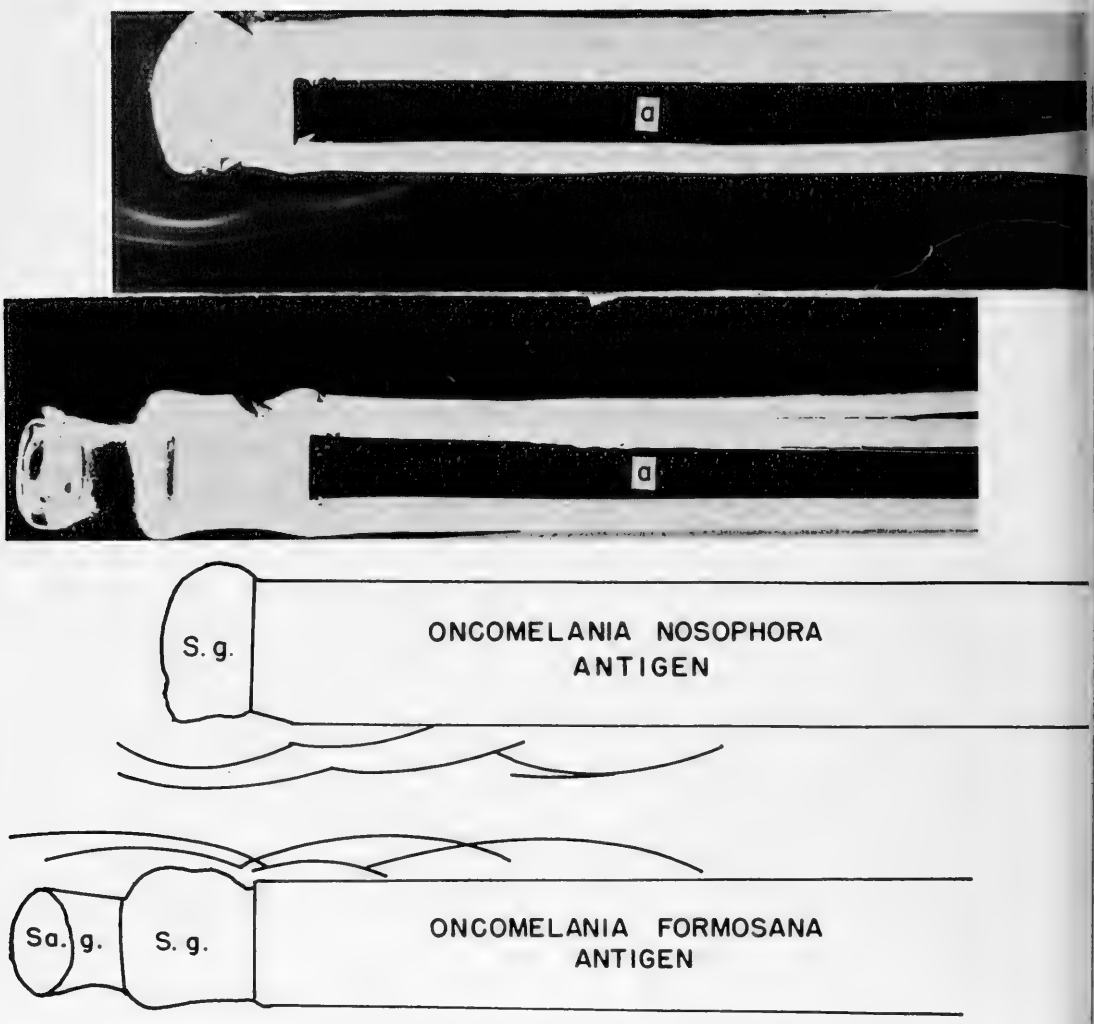
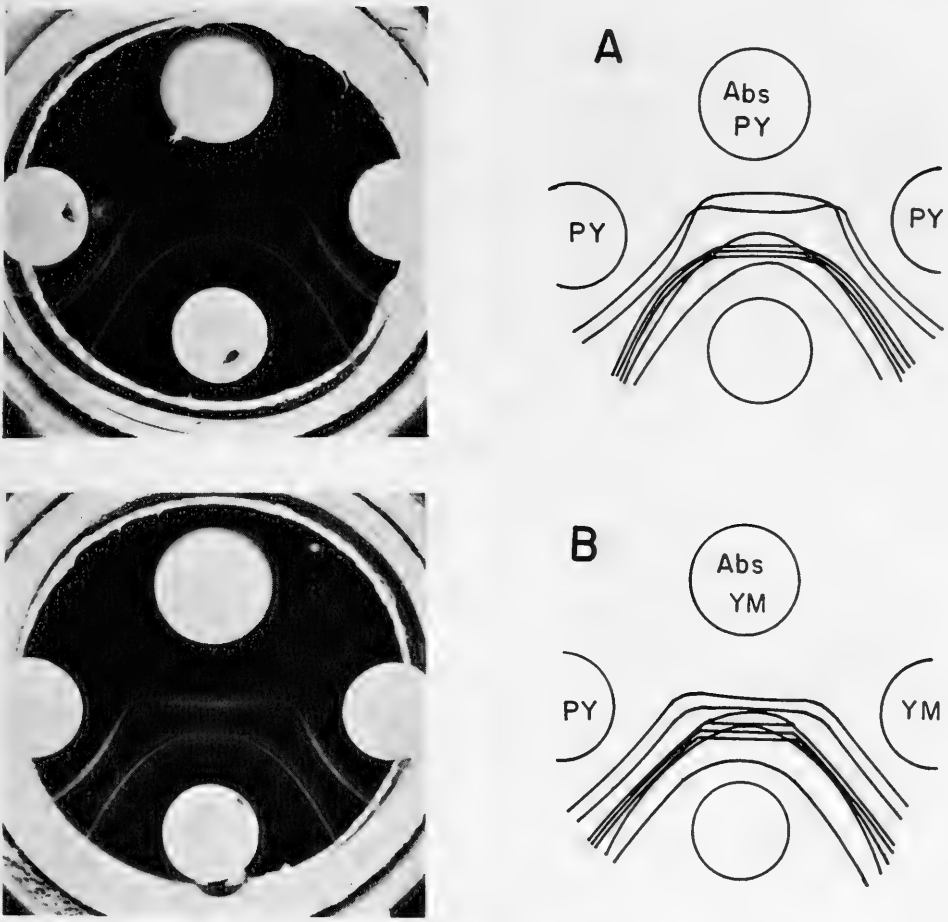


FIG. 18. Immunoelectrophoretic precipitin patterns obtained on slides with filter paper strips soaked in 5th day anti-Pu Yen serum and 2 gel columns of electrophoretically separated proteins from snail foot muscle extract. The filter paper strips have been removed; the sample gel (Sa.g.) of the upper gel column was lost prior to photographing the results. Prints of 2 slide set-ups were placed together. The antigen-antibody interference arcs, clear in the gel, did not reproduce well in the lower photographic print. The dark strips (a) over the gel columns are artifacts of reproduction occurring where the surface of the gel columns emerged above the enveloping layer of agar. The interference arcs showed visible reactions only up to component 14 (see Fig. 17). Several strongly reacting systems involved antigens present in the sample (Sa.g.) and spacer (S.g.) gels that were not part of the electrophoretically separated components.

antigens farthest from the origin of the gel column correspond to band 15 in Fig. 16B. Several antigens never enter the protein separating gel (right side, Fig. 18), but remain in the sample gel (Sa.g.) or spacer gel (s.g.).

From the long precipitin arcs showing no spurs but a slight dip (or indentation) one would assume that several separated fractions had homologous antigenicity (Fig. 18).

In the specific absorption tests, the



FIGS. 19-25. Photographs and tracings of immunodiffusion precipitin patterns using a technique of specific absorption. The homologous reactions involved *Oncomelania hupensis formosana* Pu Yen antigens (PY) (in one of the lateral wells) and 5th or 10th day anti-Pu Yen serum (top and bottom wells). Heterologous antigens (the other lateral well) were from the following: Yueh Mei (YM) and I-lan populations of *O. h. formosana; O. h. chiui* (TC); *O. h. hupensis* (OH); *O. h. nosophora* (ON); and *O. h. quadrasi* (OQ). "Abs" indicates antiserum absorbed by antigens as marked in the "Abs" well. Note opaque precipitates enlarging that well. The homologous unabsorbed antiserum was placed in the unmarked (lower) wells. The centers of the wells are 12 and 16 mm apart.

FIG. 19. Specific ansorption with homologous Pu Yen antigens (PY) in A and heterologous Yueh Mei antigens (YM) in B, using 5th day anti-Pu Yen serum, yields homologous results.

results shown in Figs. 19-23 were derived from antiserum obtained 5 days after the last injection, and those shown in Figs. 24-25 from 10th day antiserum. The latter show patterns with fewer and slightly weaker precipitation systems.

In the control (homologous or reference) reactions (anti-Pu Yen serum against Pu Yen snail foot tissue extract) the number of visable systems varied from 6-10.

As shown in Figs. 19-22, antigen-

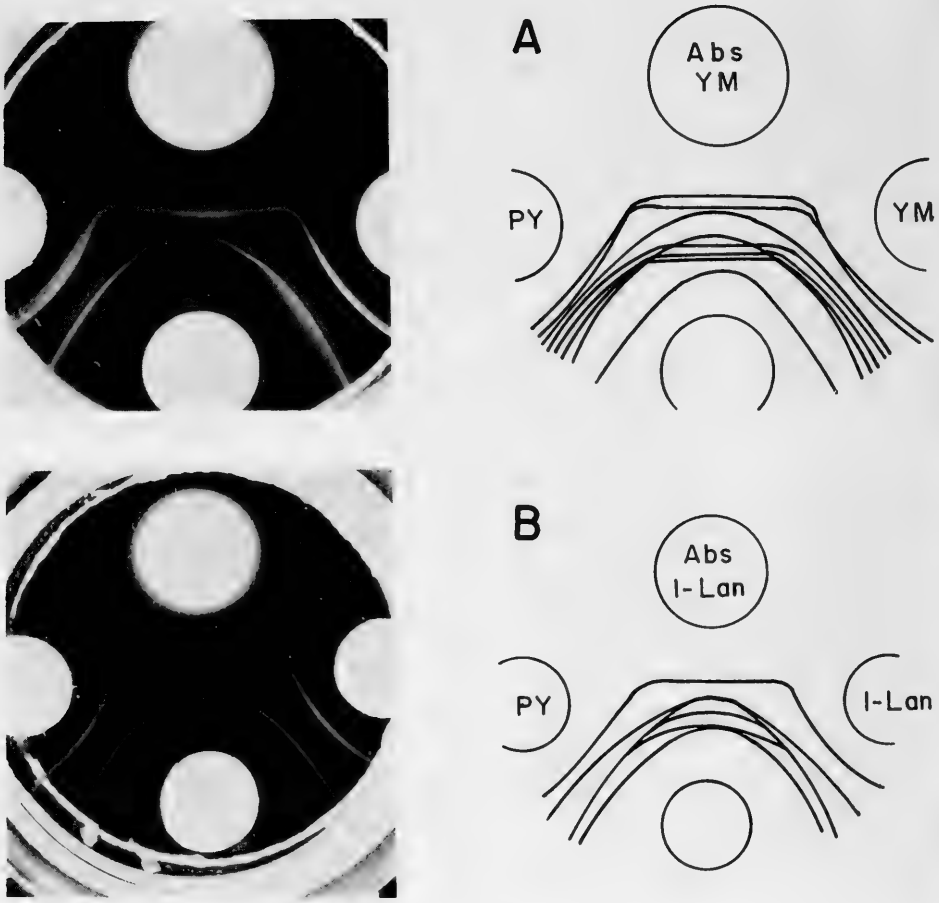


FIG. 20. Results with the heterologous Yueh Mei (A) and I-lan (B) populations of *Oncomelania hupensis formosana*, using 5th day anti-Pu Yen serum, show homologous reactions.

antibody reactions were those of identity (i.e., type 1 in system of reaction classification reviewed by Ouchterlony, 1958). The symmetry of reaction indicated that the initial concentration of antigens was similar in each of the 2 lateral wells. Homologous results were obtained with *Oncomelania hupensis formosana* from Pu Yen (control reaction; Figs. 19A, 21A), Yueh Mei (Figs. 19B, 20A) and I-lan (Figs. 20B, 22A) as well as with *O. h. chiui* (Figs. 21B, 22B). In one experiment (Figs. 22A), the reactions were not strong and one interference arc did not come to completion.

When the other subspecies of *Oncomelania* were tested it appeared that 20-40% of the interference arc systems demonstrated in *O. h. formosana* and *O. h. chiui* were not present (Table 14). As seen in Fig. 23, there were 3 distinct precipitin systems particular to *O. h. formosana* and not present, or of too low a concentration to be demonstrated, in *O. h. hupensis*. The reactions are type 3 (partial identity) in Ouchterlony's system of classification.

The reactions obtained with 10th day antiserum (Figs. 24-25) were relatively weaker, but clearly showed that *O. h. quadrasi* definitely lacked 1-2 systems that are characteristic for *O. h. for-*

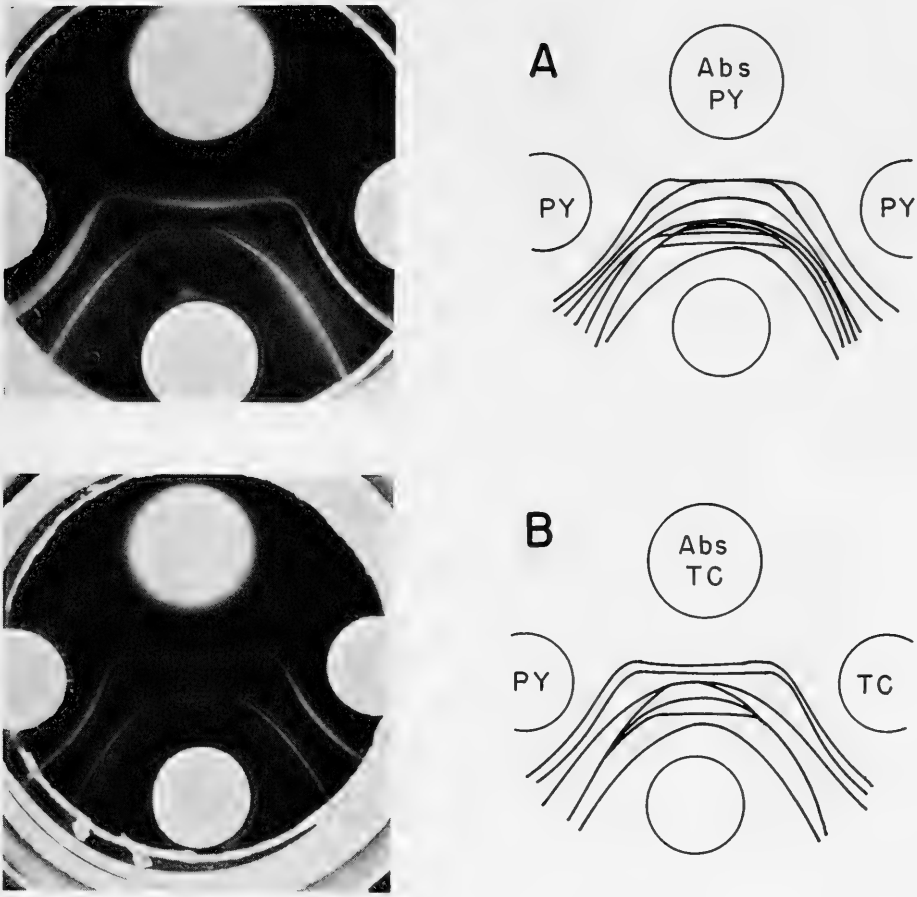


FIG. 21. Results with homologous antigens (PY) in A and with heterologous *Oncomelania hupensis chiui* antigens (TC) in B, using 5th day anti-Pu Yen serum, show no antigenic differences between the 2 taxa.

mosana, and had a reaction of both type 2 (non-identity) and type 3 (partial identity). *O. h. nosophora* equally lacked 2 systems particular to *O. h. formosana* and showed the same reaction types as *O. h. quadrasi*.

4. Discussion

Individual antigen-antibody systems serve as valid characters for a certain level of systematic comparison; it is therefore evident that, using these characters, *Oncomelania hupensis chiui* and populations of *O. h. formosana* are at the same taxon level. In terms of these immuno-diffusion systems *O. h. formosana* and *O. h. chiui* form a grouping which, on account of their antigenic properties, excludes the other subspecies of *O. hupensis*.

It is not intended here to rank the degree of difference existing in these other subspecies, but only to point out that differences do occur. Suffice it to note that the differences observed between *Oncomelania hupensis formosana*

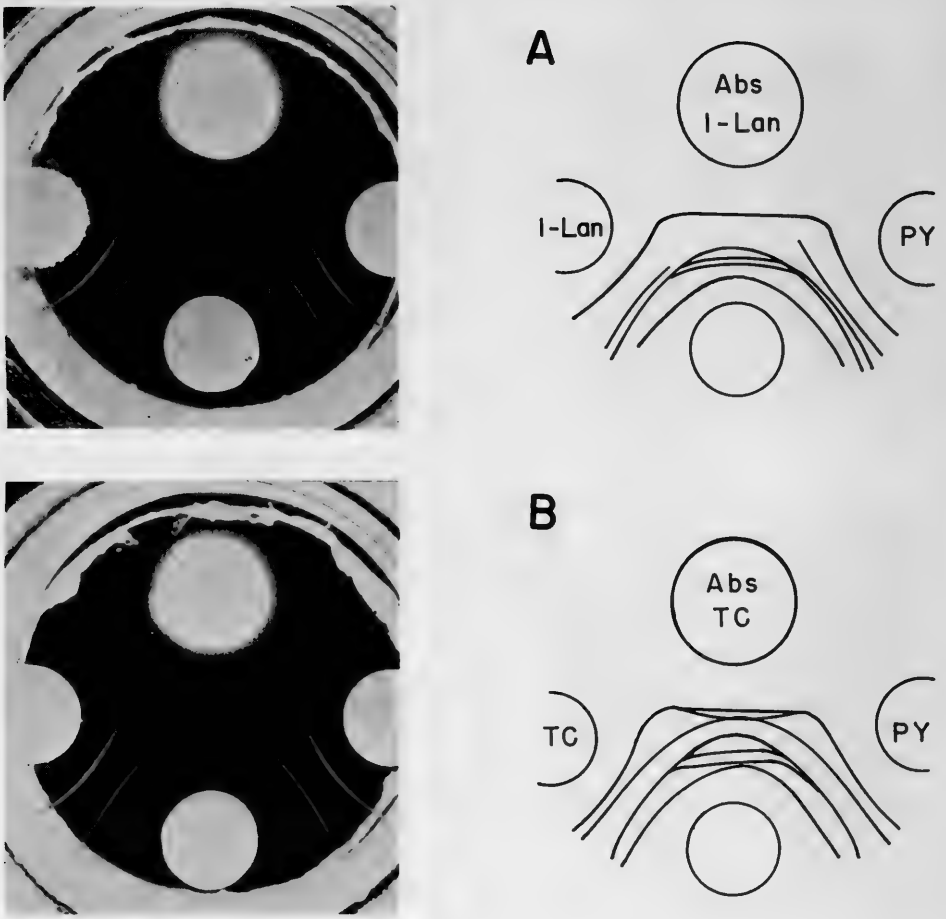


FIG. 22. Results with heterologous antigens from the I-lan populations of *Oncomelania hupensis formosana* (A) and *O. h. chiui* (B), using 5th day anti-Pu Yen serum, show no antigenic differences between the 2 taxa. In A, 1 weak precipitin arc did not completely develop in this particular reaction.

on the one hand, and *O. h. nosophora* and *O. h. quadrasi* on the other, involve the larger molecular components from the PY well (Figs. 24-25), which are slow in diffusion, while those seen in *O. h. hupensis* partly involve some of the smaller molecular weight, more rapidly diffusing components (Fig. 23), which have migrated farther from the PY well. However, differences presumably arising from the use of 5th day antiserum in the former 2 and of 10th day antiserum in the latter subspecies should not be disregarded.

In general, on strength of reaction, it appeared that *Oncomelania hupensis quadrasi* was more closely allied to *O. h. formosana* than was either *O. h. nosophora* or *O. h. hupensis* (Figs. 23-25).

The lack of demonstrable antigen-antibody systems in the fast moving fractions of the immunoelectrophoretic tests, (i.e., those from component 16 to the

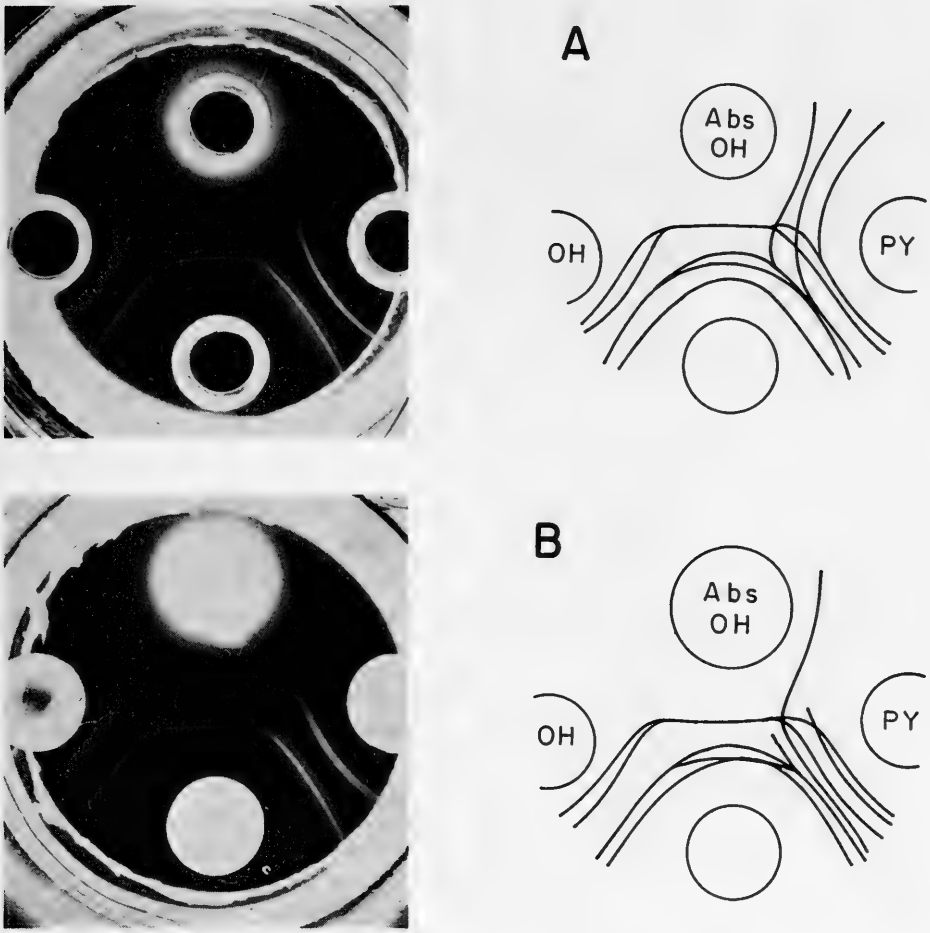


FIG. 23. Specific absorptions (A and B) both involving *Oncomelania hupensis hupensis* (OH) and Pu Yen *O. h. formosana* (PY) antigens, and 5th day anti-Pu Yen serum: 3 precipitin systems are particular to Pu Yen snails (PY).

front) was possibly due to so low a concentration of the reactants in those fractions, that visible precipitation did not occur. Other possible reasons are low concentration of those fractions in the injection series and/or poor antigenicity.

CONCLUDING DISCUSSION

Malacological

It was necessary to use an integrated approach in order to assign the taxon here investigated to each of the various category levels from subfamily to sub specific.

Characters of pedal crease, head morphology, mode of progression, ecology, egg laying, configuration of the central tooth of the radula, and general anatomy all indicated that the snail in question belonged in the hydrobiid subfamily Pomatiopsinae (Stimpson, 1865; Davis, 1966, 1967) of which *Oncomelania*, but not *Tricula*, is a member.

The genus *Tricula* (Hydrobiidae, Triculinae) is distinctly different from

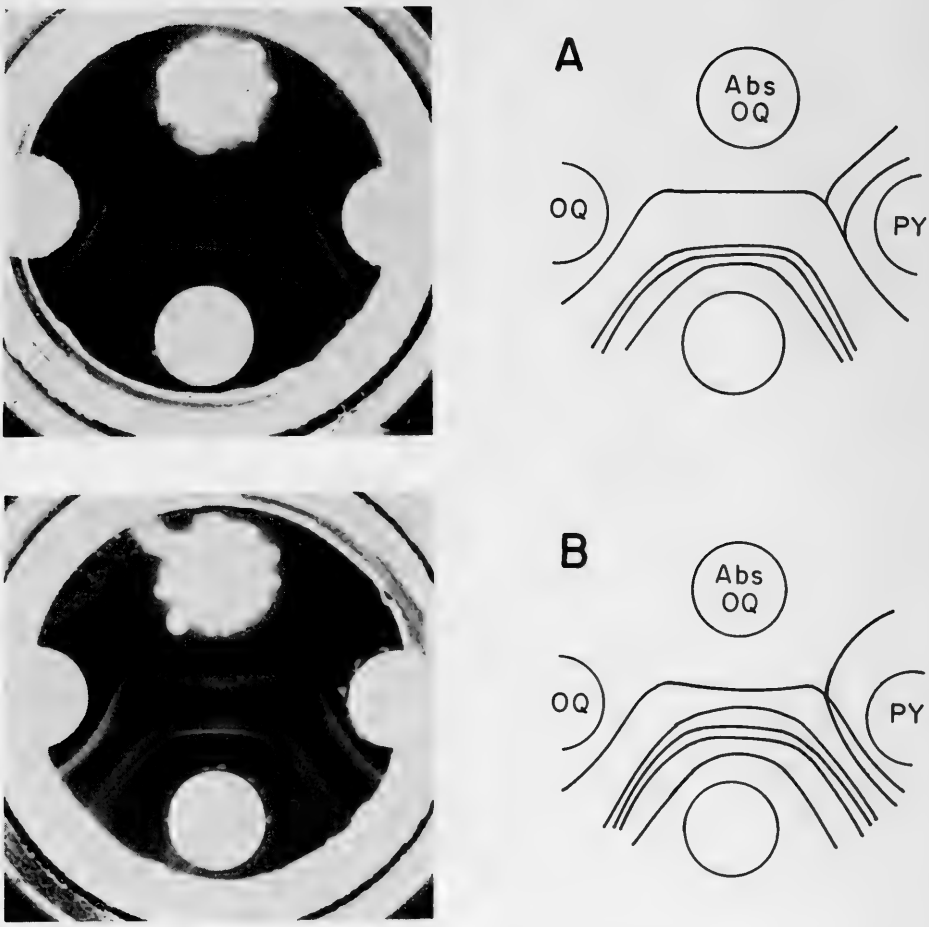


FIG. 24. Specific absorptions (A and B) both involving *Oncomelania hupensis quadrasi* (OQ) and Pu Yen *O. h. formosana* (PY) antigens, and 10th day anti-Pu Yen serum: 1-2 precipitin systems, quite weak, are particular to the Pu Yen snails (PY).

Oncomelania. Even on the basis of classical conchology the snail could not be assigned to the former genus. I have studied the types of *Tricula* from India and the Shan States of Burma which are stored at the Zoological Survey of India, Calcutta. The type of genus, *Tricula montana* Benson, was discovered by me in 1964 in the Goodwin-Austin collection at the British Museum. A vial containing 2 specimens constituted the type series. I chose one as lectotype, the other as paralectotype. The lectotype was given the accession number 1964426, the paralectotype 1964427.

On the basis of shell, species of *Tricula* vary from very small (2.4 mm, *T. gravelyi*) to moderately large (6.0 mm, *T. taylori*). On the whole they are small (*T. montana*, 3.5-3.8 mm; *T. gregoriana expansa*, 5.0 mm). By contrast the taxa included in *Oncomelania* range from 4.7-10.0 mm.

Species of *Tricula* are characteris-

FIG. 25. Specific absorption involving *Oncomelania hupensis nosophora* (ON) and Pu Yen *O. h. formosana* antigens, using 10th day anti-Pu Yen serum. One weak system was particular to the Pu Yen snails (PY).

tically glossy or white-polished when cleaned, contrasting with the opaque to translucent yellowish shell of *Oncomelania*. They lack the varix and wide umbilicus of *Oncomelania*. Most species of *Tricula* are without umbilicus or have only a slight umbilical slit.

Several species of *Tricula* are characterized by very flat-sided whorls (*T. taylori*) and have a keel at the periphery (*T. horae*, *T. horae* v. *major*), conditions not found in *Oncomelania*.

As pointed out by Rao (1928), the anterior margin of the aperture may project "considerably in advance of the outline of the last whorl" (apertural view) as in *Tricula montana*. In *Oncomelania* the anterior margin of the aperture grows in regular even symmetry with preceding whorls.

Finally, in *Tricula* the adapical end of the aperture is quite narrowed and somewhat produced (*T. montana*, *T. martini*), and the aperture is narrowly ovate to pyriform. These features contrast with the widely ovate aperture in *Oncomelania* where the adapical portion is only slightly constricted.

Habe & Miyazaki (1962) stated that the snail here identified as *Oncomelania hupensis chiui*, was an "ally" to "*Katayama formosana* (Pilsbry et Hirase)" (i.e., *Oncomelania hupensis formosana*) but that the latter "differs from the new species in having the elongate oval shell with acute apex and yellowish brown color." Actually, as shown in the discussion on the shell, when this snail is bred under optimal conditions in the laboratory, it does have the characteristics quoted for *Oncomelania hupensis formosana*. What one observes in nature is an ecophenotype in which the nature of the environment causes extreme erosion and wear on the shell to the extent that the thickened outer lip does not develop and shells appear very short and globose.

A parallel case is seen in the habitat of *Tricula minima* (= *Fukuia kikuchi* Kuroda) on Sado Island off Honshu, Japan. Snails similarly living on a steep gradient in trickling water also had extremely

TABLE 14. Proportion of congruent antigen-antibody systems in various subspecies of *Oncomelania hupensis* as compared to those found in *O. h. formosana* from the Pu Yen area by immuno-diffusion, using the specific absorption technique (compare with Figs. 19-25)

Subspecies of *Oncomelania hupensis*	Area of origin	Precipitin systems found %
	Taiwan*	
formosana	Pu Yen	100
formosana	Yueh Mei	100
formosana	I-lan	100
chiui	A-li-lao	100
quadrasi	Philippines**	66-83
hupensis	China**	63
nosophora	Japan**	60

* Compare with Fig. 1.

** from field collected or laboratory stocks of snails from these countries.

eroded shells, appeared badly worn, and were short and globose.

The internal anatomy of *Oncomelania hupensis chiui* is strikingly similar to that of *Oncomelania hupensis formosana*. Therefore, on the basis of both shell and anatomy, the taxon is assigned to the genus *Oncomelania*. Creation of a separate genus is certainly not warranted nor should it even be considered. Only much larger anatomical differences would justify such a decision. Examples of such differences are given by Davis (1967), who discusses separate generic status for *Oncomelania* and *Pomatiopsis* (the latter genus being the one most closely related to *Oncomelania*). The anatomy of *Tricula* (Davis, MS) shows major important differences, which places the genus in the hydrobiid subfamily Triculinae.

There are numerous detailed anatomical similarities of structure between the snail discussed and *Oncomelania hupensis formosana* and 5-6 more important differences. The smaller size, reduced number of gill lamellae, slight varix, greater size of certain nervous structures, and differences in frequency of certain denticular counts on the radula are, however, not considered sufficient to justify full specific status, for the following reasons: 1) The above mentioned overwhelming number of similar structures and substructures between *O. h. chiui* and *O. h. formosana*. 2) The complete homogeneity in the characters of antigen-antibody systems between the snail in question and different populations of *O. h. formosana*, even though homogeneity was not as complete for the other subspecies of *Oncomelania hupensis*. When all subspecies were tested in a similar manner, it was apparent that *O. h. formosana* had unique systems either not present in the other subspecies, or of very low density. 3) *O. h. chiui* will mate with *O. h. formosana* and produce fertile hybrids. The production rate for young (hybrids) is the same as that for *O. h. formosana*. 4) The electrophoretic densitometric pattern of *O. h. chiui* clearly fits the pattern for I-lan *O. h. formosana;* in particular it closely resembled the church population. The resemblance in patterns is decisive, particularly as *O. h. formosana* from Pu Yen had a different densitometric pattern indicative of a definite population difference.

For these various reasons the taxon is assigned subspecific rank under *Oncomelania hupensis*. On the basis of electrophoretic pattern it would appear that *O. h. chiui* owed its origin to that stock which also gave rise to present day I-lan snails. As seen in Fig. 1, the localities of A-li-lao and I-lan are rather close to one another, in comparison to the Pu Yen and Yueh Mei

areas; however, they are separated by extremely high and rugged mountains which rise rather abruptly from the sea. I-lan county is, itself, quite an isolated pocket.

Taiwan is characterized by such isolated habitats which provide the segregation necessary for conservation of local genetic change and eventual speciation.

Parasitological

After Dr. Chiu and I had initially recognized that *"Tricula chiui"* closely resembled *Oncomelania*, the snail intermediate host of *Schistosoma japonicum* in the Far East, this relationship was further confirmed parasitologically by successful infection of that snail with the Formosan, solely zoophylic strain of *S. japonicum* (Chiu, 1965b), which is endemic in central western Taiwan, Chang-hua county.

Moreover, the anatomical and biophysical studies described in this paper have not only indicated the snail first described as *"Tricula"* to be a subspecies of *Oncomelania hupensis*, but to be most closely related to *O. h. formosana*, and, in particular, to a population of that subspecies from I-lan county, also located in the northern tip of Taiwan.

The first indication of susceptibility of Formosan *Oncomelania hupensis formosana* to human schistosomes was given by Moose & Williams (1964a, b), who succeeded in infecting I-lan snails with schistosome strains from the Philippines and Japan. These findings suggested that *O. h. chiui* might also be susceptible to infection with human strains of *S. japonicum*. Chiu (1967) has indeed shown that the snail can be infected with the Japanese strain of the parasite.

These recent discoveries are of considerable epidemiological interest. No longer does Hsü & Hsü's (1962) statement hold that: "since oncomelanian snails in Formosa are not susceptible to imported strains from Japan or the Chinese mainland, this may be the reason - a fortunate reason - why the human strain was not able to establish and complete its life cycle on Formosa." There is now no doubt, as Moose & Williams (1964b) stated, that "oncomelanid snails susceptible to infection with human strains of *Schistosoma japonicum* do occur on the island of Taiwan."

Recent investigations of the diverse susceptibility of geographical races of *Oncomelania hupensis formosana* to various schistosome strains (Moose & Williams, 1963, 1964b) reveal that the greatest complexity of snail parasite-relations is to be found on Taiwan. The distinct subunits of *Oncomelania* are just beginning to be studied in relation to presence, complexity and relationships. They vary in terms of slightly different ecology, somewhat different general appearance, and vastly different susceptibilities. In the light of these fairly new epidemiological findings and the biological data presented here, it appears that Taiwan provides the main focal point for studying and establishing the underlying causes for the complex shifts in genetic potential discussed in the literature as "strains" of *S. japonicum*. Likewise to be pointed out is the fact that data derived from malacological studies are of value in predicting, to a degree, the relationship of a snail taxon and its susceptibility to parasitic involvement.

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RÉSUMÉ

UNE ETUDE SYSTEMATIQUE D'*ONCOMELANIA HUPENSIS* CHIUI (GASTROPODA: HYDROBIIDAE)

G. M. Davis

Le gastropode Hydrobiidae que Chiu (1961) signala être le premier hôte de *Paragonimus iloktsuenensis* à Formose fut décrit comme *Tricula chiui* par Habe et Miyazaki (1962). Le gastropode, trouvé dans un bassin isolé du Nord de Formose (région d'A-li-lao), se révéla plus tard susceptible de transmettre aussi *Schistosoma japonicum* (Chiu, 1965b, 1967). Des observations anatomiques ont désormais montré que ce gastropode appartient au genre *Oncomelania*. Il est très proche des sous-espèces de *O. hupensis*, décrites sour les noms de *O. h. formosana* (Davis, 1967), *O. h. nosophora* et *O. h. quadrasi* (Davis, non publié), parceque: 1) la verge porte une papille et des bandes cilées caractéristiques près de la pointe; 2) la vésicule séminale forme un "noeud"; 3) chez la femelle, le canal de la spermathèque et le "canal du sperme" apparaissent sur le côté droit de la poche copulatrice, près de l'extrémité antérieure, comme deux tubes séparés mais entourés d'une enveloppe commune de tissu conjonctif; 4) l'oviducte s'enroule autour du réceptacle séminal de façon distincte; 5) la structure des glandes mâle et femelle est semblable; 6) la coquille est typiquement d'un *Oncomelania* malgré la varice (bourrelet creux) peu marquée.

Des études d'immunologie, utilisant la méthode d'Ouchterlony avec double diffusion d'antiserum (anti-muscle pédieux d'*O. h. formosana*), ont indiqué que *"Tricula chiui"* est plus proche de *O. h. formosana* que de toute autre sous-espèce de *Oncomelania hupensis*, car: 1) tous les systèmes antigene-anticorps sont homologues entre eux; 2) ils ont des systèmes qui ne sont pas présents chez les autres sous-espèces de *O. hupensis* ou qui n'y sont que partiellement identiques.

L'électrophorèse sur polyacrylamide des protéïnes extraites du muscle pédieux de *"T. chiui"* et de plusieurs populations de *O. h. formosana*, ont montré que la courbe de densité optique de *"T. chiui"* est plus proche de celle d'une population de *O. h. formosana* du Nord-Est de Formose (région d'I-lan), que d'aucune autre.

Des études d'hybridation ont montré que *"T. chiui"* produit des hybrides fertiles avec *O. h. formosana*.

En tenant compte de ces observations il apparait évident que le gastropode originellement nommé *"Tricula chiui"* a tiré son origine d'un stock de *O. h. formosana* normal de la région d'I-lan et qu'à la suite de son isolement en bordure de mer à A-li-lao, que de hautes montagnes séparent de la région d'I-lan, ce stock a acquis de nouvelles caractéristiques qui justifient le statut de sous-espèce, à savoir, 1) une coquille plus courte, 2) un nombre de filaments branchiaux significativement plus faible que chez les autres espèces de *O. hupensis*, 3) une coquille avec une varice obsolète, 4) un connectif pleurosupraoesophagien plus long que chez *O. h. formosana*, 5) un plus grand ganglion supraoesophagien, 6) une différence dans la fréquence du nombre de cuspides sur les dents de la radula. En conséquence le taxon est nommé *Oncomelania hupensis chiui*.

RESUMEN

ESTUDIO SISTEMATICO DE *ONCOMELANIA HUPENSIS CHIUI* (GASTROPODA: HYDROBIIDAE)

G. M. Davis

El caracol hidróbido que Chiu (1961) registró como el primer huéspued intermediario de *Paragonimus iloktsuenensis* en Taiwan (Formosa) fué descripto como *Tricula chiui* por Habe & Miyazaki (1962). Este caracol, encontrado en una cuenca aislada del norte de Taiwan (Ai-li-lao area), posteriormente demostró ser susceptible a *Schistosoma japonicum* (Chiu 1965b, 1967). Sus detalles anatómicos muestran ahora que pertenece al género *Oncomelania*. Es muy afín a la subespeciesde *O. hupensis*, descriptas como *O. h. formosana* (Davis, 1967), *O. h. nosophora*, y *O. h. quadrasi* (Davis, sin publicar), porque: 1) la verga tiene papila y caracteristica banda ciliar cerca de la punta; 2) la vesícula seminal es nudosa; 3) la espermateca y ducto espermático salen del borde lateral derecho de la bolsa copulatriz cerca del extremo anterior, como un par de tubos separados pero envueltos en una vaina común de tejido conjuntivo; 4) el oviducto se arrolla sobre el recepetaculo seminal en una forma particular; 5) la estructura de las gonadas maculina y femenina son similares; 6) la concha es distintamente del tipo de *Oncomelania* a pesar del poco desarrollo de la várice.

Estudios immunológicos usando micro-Ouchterlong en pruebas de doble difusión antisuero (anti- *O. h. formosana*, extrato del musculo pedal), indicaron que *"Tricula chiui"* estaba asociada más estrechamente con poblaciones de *O. h. formosana* que con las otras subespecies de *Oncomelania hupensis* porque: 1) todos los sistemas de antigenos-anticuerpos eran homólogos entre ellos; 2) tenian sistemas no presentes, o sólo parcialmente iguales, con aquellos que aparecen en otras subespecies de *O. hupensis*.

Electroforesis poliacrimalida de proteinas extractadas del musculo pedal de *"T. chiui"* y varias poblaciones de *O. h. formosana* mostró que la primera tiene un perfil densitométrico de los componentes proteicos separados, más similar a una población de *O. h. formosana* del N. E. de Taiwan (I-lan distrito) que a cualquier otra.

Estudios de hybridización mostraron que *"T. chiui"* produjeron híbridos fertiles con *O. h. formosana*.

Por la reunión de estos datos, parece como muy probable que el caracol originalmente llamado *"Tricula chiui"* tuvo su origen en el linaje que dió lugar al surgimiento de la corriente *O. h. formosana* de I-lan, y que el aislamiento que siguió cerca del borde maritimo en A-li-lao, donde altas montañas separa esta region de la I-lan, produjo caracteristicas que justifican el status subespecifico, como ser, 1) conchilla mucho más corta, 2) significativamente menor número de filamentos branquiales que en las otras subespecies de *O. hupensis*, 3) conchilla con várice rudimentaria, 4) un conectivo pleuro-supraefágico más largo que en *O. h. formosana*, 5) ganglio supraesofágico más grande, 6) diferencia en frecuencia de número de cúspides en los varios dientes radulares. A acuerdo con todo esto el taxon debe llamarse *Oncomelania hupensis chiui*.

АБСТРАКТ

ИЗУЧЕНИЕ СИСТЕМАТИКИ *ONCOMELANIA HUPENSIS CHIUI* (GASTROPODA: HYDROBIIDAE)

Г. М. ДЕВИС

Гидробииды, которые по Чиу (1961) являются первыми промежуточными хозяевами *Paragonimus iloktsuensis* на Тайване, были описаны Хабе и Миязаки (1962) как *Tricula chiui*. Эти моллюски, найденные в изолированном бассейне на Северном Тайване (район Аи-ли-ляо), как было установлено позже, также восприимчивы к *Schistosoma japonicum* (Чиу, 1965б, 1967).

Судя по анатомическим данным, было выяснено, что этот моллюск относится к *Oncomelania*. Он близко родственен к подвиду *O. hupensis*, как это было описано для *O. h. formosana* (Дэвис, 1967), для *O. h. nosophora* и *O. h. quadrasi* ((Дэвис, неопубликовано), потому что: 1) по краю имеются папиллы и характерная кайма ресничек у верхушки; 2) семенной пузырек узловатый; 3) сперматеки и семепротоки отходят от правого переднего бокового края совокупительной сумки ввиде двух отдельных трубок, связанных между собой общей соединительнотканной оболочкой; 4) яйуепровод образует характерный изгиб над семенным пузырьком; 5) строение мужской и женской гонад сходно; 6) раковина типа *Oncomelania*, несмотря на отсутствие varix.

Иммунологическое исследование при помощи тестов двойной диффузии методом микро-Аухтерлонга с адсорбцией антисерума (как анти - употреблялся экстракт из ножного мускула *Oncomelania hupensis formosana*), показало, что *"Tricula chiui"* стоит ближе к популяциям *O. h. formosana*, чем к другим подвидам *O. hupensis*, потому, что: 1) все системы антиген-антител гомологичны между собой; 2) имеются системы, не представленные или лишв частично идентичные тем, которые встречаются у других подвидов *O. hupensis*.

Полиакриламидный электрофорез белков, экстрагированных из ножного мускула *"Tricula chiui"* и некоторых популяций *Oncomelania hupensis formosana* показало, что первая имела денситометрические профили отдельных компонентовв белков более сходные 1 популяции *O. h. formosana* из северо-восточного Тайваня (район Ай-лян), чем к каким-нибудь другим.

Гибродизация показала, что *"Tricula chiui"* давала с *Oncomelania hupensis formosana* плодовитое потомство.

Из всех этих данных следует, что моллюски, первоначально названные *"Tricula chiui"* вероятнее всего произошли от *Oncomelania hupensis formosana* из района Ай-лян; последующая изоляция у берега моря в районе Ай-ли-

ляо, где высокие горы отделяют их от района Ай-лян, привела к тому, что эта популяция приобрела черты, придавшие им ранг подвида, а именно: 1) гораздо более укороченная раковина; 2) значительно меньшее количество жаберных нитей, чем у других подвидов *O. hupensis*; 3) раковина без varix; 4) плевро-надглоточная коннектива более длинная, чем это имеется у *O. h. formosana*; 5) более крупный надглоточный ганглий; 6) различия в частоте и количестве зубцов на различных зубах радулы.

В соответствии с этим данная форма получила название ***Oncomelania hupensis chiui***.

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STUDIES ON THE REPLACEMENT OF THE GASTROPOD RADULA

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ABSTRACT

Radulae were marked at their posterior end by: cautery, magnesium chloride injection, colchicine injection, or cold shocking. Then the forward movement of the marked area was used to determine the occurrence, and rate, of radular replacement. The new techniques of magnesium chloride and colchicine injection made marking of the radula possible without lengthy operation, and cold shocking allowed marking of the radulae of small species and young animals which could neither be operated on nor be injected because of their small size. The various methods used for marking yielded comparable results. Continuous replacement was found in all prosobranchs and pulmonates examined: *Littorina saxatilis, L. littorea, L. littoralis, Patella vulgata, Thais lapillus, Buccinum undatum, Viviparus viviparus, Pomatias elegans, Agriolimax caruanae, A. reticulatus, Arion ater, Helix aspersa, Cepaea nemoralis, Lymnaea stagnalis, Achatina fulica.*

There is a very rapid rate of replacement of the radula in newly hatched animals followed by a steady decrease in replacement rate. In older animals this rate varies with shell size, being slower in larger animals. The rate of replacement is proportional to temperature.

When the posterior part of the radula is destroyed, loss of the old teeth at its anterior end occurs at a rate similar to that of normal radula production.

During aestivation the rate of formation of new radula was the same as in controls, during hibernation it was somewhat slower. Movement forwards was, however, slower than normal, resulting in corrugation of the radula over the collostyle. Old teeth did not become separated but accumulated at the front of the radula.

The rate of secretion of new teeth, in auto- and homografts of the posterior part of the radula into the haemocoel, was similar to that in the whole radula: in heterografts between young related animals, replacement was slower than normal, and in heterografts between old or less closely related animals no replacement occurred.

INTRODUCTION

That the molluscan radula must be continually replaced was established by studies on (1) the histology of the radular gland (Rossler, 1885; Schnabel, 1903; Pruvot-Fol, 1925, 1926; Hoffman 1932; Märkel, 1957; Runham, 1963); (2) the growth of the radula (Sterki, 1893; Thompson, 1958; Hubendick, 1945); and (3) through observations of the occurrence of discarded radula teeth in the faeces (Geyer, 1927; Carriker, 1943; Quick, 1960; Kühnelt, 1961). Runham (1962) was able to produce direct evidence for a continual replacement of the radula in *Lymnaea stagnalis*.

Radula replacement is therefore a type

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of 'continuous physiological regeneration' (Vorontsova & Liosner, 1960). Examples of such regeneration have been only poorly investigated in invertebrates, except for recent work on the teeth of echinoderms (Holland, 1965), and the factors affecting it have been almost completely neglected. Previous work on the gastropod radula has produced but indirect evidence for changes in the rate of radula replacement due to age and size (Hubendick, 1945; Thompson, 1958)

This paper reports further studies on the continuous replacement of the radula in a total of 15 prosobranch and pulmonate species and contributes direct evidence for the effects of temperature, size, age, dormancy, partial removal, and transplanting, on replacement.

MATERIALS AND METHODS

Source of animals

All the animals were collected locally in North Wales except for *Viviparus viviparus* which were obtained from L. Haig and Co., Surrey, and the tropical *Achatina fulica* which were a generous gift from Dr. R. H. Nisbet, Department of Physiology, Royal Veterinary College, London.

Maintenance of animals

The terrestrial molluscs were kept in plastic bowls covered with a glass sheet, with a bowl of water on the bottom, some moist "sphagnum" moss and some cuttlefish bone as a source of calcium. *Pomatias elegans* were kept on moist calcareous soil collected from their normal habitat. The relative humidity in the bowls was in excess of 90%. To obtain dormancy, *Helix aspersa* were kept without water and the bowls were covered with gauze instead of glass. The humidity in the bowls then was the same as in the laboratory (60-62% relative humidity). The marine and freshwater species were kept in plastic bowls in enough water to cover them, together with some plants and a few pebbles. Due to lack of facilities the marine species had to be kept in still water which was changed daily. Animals were originally kept in the laboratory at temperatures of 10-20° C except for *Achatina fulica* which were kept at 20-25° C. In later experiments it was found necessary to maintain animals at constant temperatures. The bowls were then placed in constant temperature cold rooms at 0 ± 2° C and 10 ± 1° C, in constant temperature water baths in the cold rooms at 5 ± 0.1° C and 15 ± 0.1° C and in a hot air oven at 20 ± 0.5° C.

The terrestrial species were fed on sliced raw carrot and lettuce. The freshwater forms were supplied with *Elodea canadensis*, *Potamogeton obtusifolia*, filamentous algae and lettuce, which they usually preferred. *Littorina* spp. were provided with seaweed, i.e. *L. saxatilis* and *L. littorea* with *Fucus vesiculosus*, *F. serratus* and *F. ceranoides* and *L. littoralis* with *Pelvetia canaliculata*. Barnacles and mussels were provided for *Thias lapillus* and *Buccinum undatum*, but these carnivorous species, as well as *Patella vulgata*, which was supplied with a wide variety of algae, took very little food in the laboratory.

The food and water were renewed daily, except for dormant *Helix aspersa*, which were provided with neither. *Helix aspersa* were usually pulled off the walls of their container daily so as to prevent lengthy periods of aestivation, but animals required in aestivation or hibernation were left undisturbed.

In order to mark snails that were studied individually, the body whorl was cleaned and dried, then numbered with waterproof white ink (Pelikan). As this ink wore off rapidly the animals were checked daily and re-marked if necessary. In later experiments ink

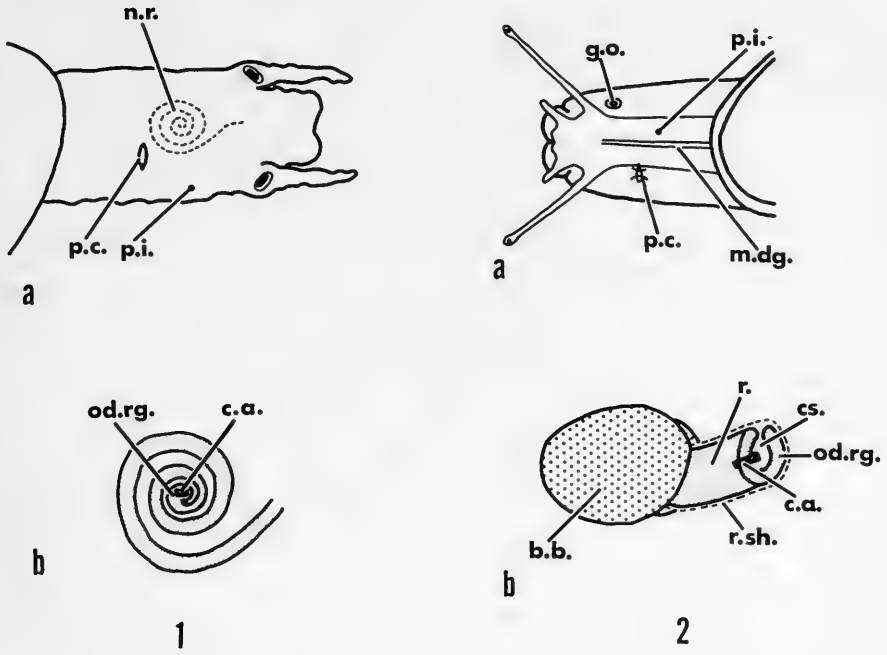

FIG. 1. A. Dorsal view of the head of *Littorina littorea* showing the positions for injection and incision. B. The radula enlarged showing the area cauterized. c.a., cauterized area; n.r., normal position of the radula; p.c., position of incision; p.i., position of injection; od. rg., odontoblast region.

FIG. 2. A. Dorsal view of the head of *Helix aspersa* showing the positions for injection and incision. B. The buccal bulb and the radula showing the cauterized area. b.b., buccal bulb; c.a., cauterized area; cs., collostyle; g.o., genital opening; m.dg., mid-dorsal grooves; od.rg., odontoblast region; p.c., position of incision; p.i., position of injection; r., radula; r.sh., radular sheath.

marker (Colorite) was found to be better for the aquatic, but not for the terrestrial forms. Slugs could not be thus marked and had to be kept individually in marked bowls. No marking was necessary when animals were treated in large numbers and maintained under identical conditions. *Arion ater* could not, however, be kept in large numbers because of its cannibalistic habits.

Marking of the radula

The posterior region of the radula was marked by 3 methods:

a. By operation and cautery. The technique of cautery was that used by Runham (1962). The techniques of anaesthesia with magnesium chloride injection are detailed in Runham, Isarankura & Smith (1965). The survival rates given

in that paper were high for *Helix aspersa* and *Littorina littorea* (77-92%), moderately satisfactory for *Arion ater* (37.5%) and poor for *Thias lapillus* and *Achatina fulica* (20-23%). The operation took place immediately after anaesthesia. The animals were placed with the left side of the head uppermost on an expanded polystyrene trough and the operations were performed under a stereoscopic microscope. It was necessary to hold down *Arion ater* by covering the mid part of the body with tissue paper and pinning it down firmly, which served 2 functions: it prevented movements due to contractions of the posterior part of the body and helped to keep the head extended.

The operating technique used depended upon the morphology of the radula gland. When operating on *Littorina littorea* the shell was held in the left hand, the operculum was gripped by a clothes peg and held down out of the shell with the same hand. A transverse incision of 1-1.5 mm was then made in the skin on the right dorsal side of the head about 8-10 mm behind the base of the right tentacle (Fig. 1A, p.c.) with a small piece of razor blade held in a needle vice. The dorsal part of the body was then pressed gently in order to move the radular coil near to the opening. When the tip of the radular gland (Fig. 1B, c.a.) appeared in the opening it was given a very brief touch with a fine platinum cautery loop. On pressing the body wall, the radular coil moved to its normal position (Fig. IA, n.r.). As the incision was so small, no suture was necessary.

In *Helix aspersa* and *Arion ater* a transverse incision about 2 mm wide was made with fine scissors in the body wall about 5 mm behind the left optic tentacle. The tip of the radular sheath was gripped with fine forceps and pulled to the opening (Fig. 2A, p.c.). A small area, where the odontoblasts are localized (Fig. 2B, od. rg.) was then cauterized (c.a.). The incision was transversely stitched (Fig. 2A, p.c.) with sterilized silk suture thread (Ethicon FM500) with a 16 mm shank threaded cutting needle.

The operation lasted about 3-5 minutes. In *Helix aspersa* the silk thread usually disappeared 3-5 days after operation, when the wound appeared completely healed and only a clear whitish scar (without tubercules) was left. In *Arion ater* the wound in the very thin extensible skin of the head rarely healed and the suture thread was usually lost. When the body contracted, the internal organs were extruded, leading to a very high mortality (about 80%). In *Littorina* the wound was usually blocked by the salivary gland and healing was complete about 10-15 days after the operation. The operated animals were normally feeding on the day after the operation.

b. By injection. Apart from the marks of cautery the radulae of operated animals were observed to have a transverse row of abnormal teeth that had arisen at the time of operation. It was found that anaesthesia by injection of magnesium chloride into the haemocoel caused this abnormality and that it could be used for marking. However, magnesium chloride injection could not be used for *Achatina fulica* or *Lymnaea stagnalis* due to its very high toxicity to these forms. As the production of the radula is reported to be dependent on cell division in the radular epithelia (Runham, 1963), injections of colchicine, a mitotic inhibitor, (0.2-0.3 ml of a 50 mg solution in distilled water) were also successfully used for marking.

c. By cold shock. Radular abnormaities that must have occurred some time

before the operation were observed in *Helix aspersa*, especially after hibernation. It was thought that these were caused by the cold winter conditions, which was later proved to be true. These abnormalities could be induced experimentally by a short cold shock. This method proved to be a very useful and simple way of marking the radula in all species, particularly those that were difficult or impossible to operate on due to size or lack of an efficient anaesthetic method. The animals were transferred from their normal conditions to a bowl previously cooled to $0^{\circ} \pm 1^{\circ}$ C. Young *Helix aspersa* were left there for 24 hours and all other species for 48 hours. *Achatina fulica* was shocked at 10° C. Temperatures lower than -1° C were fatal to most species.

Of the 3 methods, the operative one was the most reliable, because the other methods produced recognizable abnormalities only in 80-95% of animals. In some animals this was due to confusion with pre-existing abnormalities.

Transplants

In order to study if the radula would be secreted and move forwards normally when separated from the anterior part of the radula, where the teeth are being used and lost, transplants were made of the posterior part of the radula gland. The possibility of a foreign tissue reaction affecting replacement of the radula was also studied in homografts and heterografts.

Donor animals were operated on and the radula gland cauterized to mark the radula. The posterior part of the radula, comprising about 30-40 rows, the odontoblast region, and part of the collostyle, was then cut off with scissors. In contrast to the anterior portion of the radula, this posterior portion of the radula is not attached to its underlying epithelium. The transplant was then left free in the animals own haemocoel (autograft) or was transplanted into the haemocoel of another individual of the same species (homograft) or of a different species (heterograft). On dissection the grafts were usually found free in the haemocoel but sometimes they had become fused to visceral organs by thin membranes or were covered by a jelly-like material (encapsulation).

Measurements

The shell heights and diameters were measured at the end of the experiments with a vernier caliper for large shells and graph paper for small. Slugs were measured after fixation (half contracted).

Preparation of the radula

The method used was an adaptation of that devised by Thompson (1958). The animals were dropped into boiling water to kill them, then the buccal mass was dissected out and boiled in 10% potassium hydroxide until all the flesh had dissolved. The radula was removed, washed in 70% alcohol, then spread and flattened on the slide with a small brush. The mounting medium, polyvinyl lactophenol (obtained from G. T. Gurr), containing sufficient Lignin Pink to give a dark red colour, was then added and a coverslip pressed down to ensure the flat mount that is essential for counting. Long radulae (*Littorina* and *Patella*) were cut into 2 halves and these were mounted together. Owing to their height, the radular cusps had to be brushed off in these 2 species before mounting. It was found that unmounted radulae or whole animals could be stored for a long time without hardening in 70% alcohol.

The number of rows of teeth was


a
b
c
d

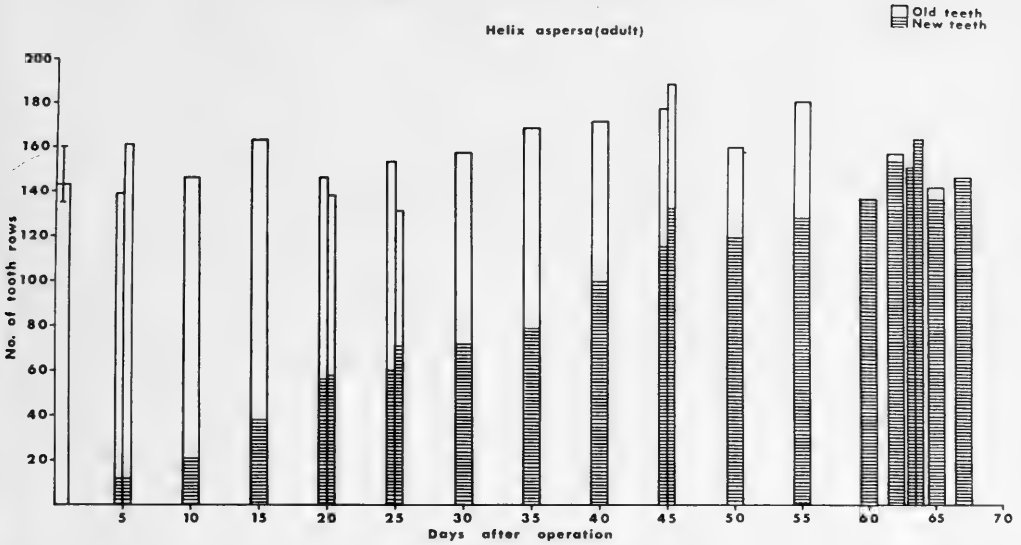

FIG. 4. Tooth replacement in adult *Helix aspersa*. Temperature 10^{o} - 20^{o} C (fluctuating). Each column represents the radula of 1 animal except for the control column (left) which is the average of 10 radulae (maximum and minimum is shown by the barred line). The shaded part of each column indicates the number of rows of teeth in the radula that were secreted after cautery and the unshaded part, rows that were present at the time of the operation.

counted twice and the rate of replacement was determined by dividing the number of rows formed since marking by the duration in days (5-250) of the experiment.

EXPERIMENTS AND RESULTS

Comparison of replacement rates after marking the radula

When a small area at the posterior end of the radula was cauterized in the way described above, some of the odontoblasts and the radular epithelia in their close vicinity were destroyed. The non-cauterized areas were unaffected and continued to secrete new radula material. It was thus very easy to determine in these experiments the number of rows of teeth produced after the operation. Results of such an experiment with *Helix aspersa* are shown in Figs. 3 and 4. The snails used were mature (shell height 2.6 - 3.0 cm) and the temperature fluctuated between 10 and 20^{o} C. Radulae were examined every 5th day up to the 65th day after marking. The average rate of secretion of the radula in 21 snails was 2.45 rows a day. It can be seen that, as the new teeth are continuously added to the radula, the old teeth are lost at a similar rate. There was, however, a very slight lengthening of the radula during the experiment. Later experiments showed that this was due to an accumulation of old teeth associated with reduced feeding activity.

FIG. 3. Radulae of adult *Helix aspersa*. The anterior portion of the radula points to the bottom. The cautery scar advances from the top during growth.

a. Normal.
b. 10 days after operation.
c. 40 days after operation.
d. 62 days after operation (cautery at the centre of the odontoblasts has caused separation of the radula into 2 halves).

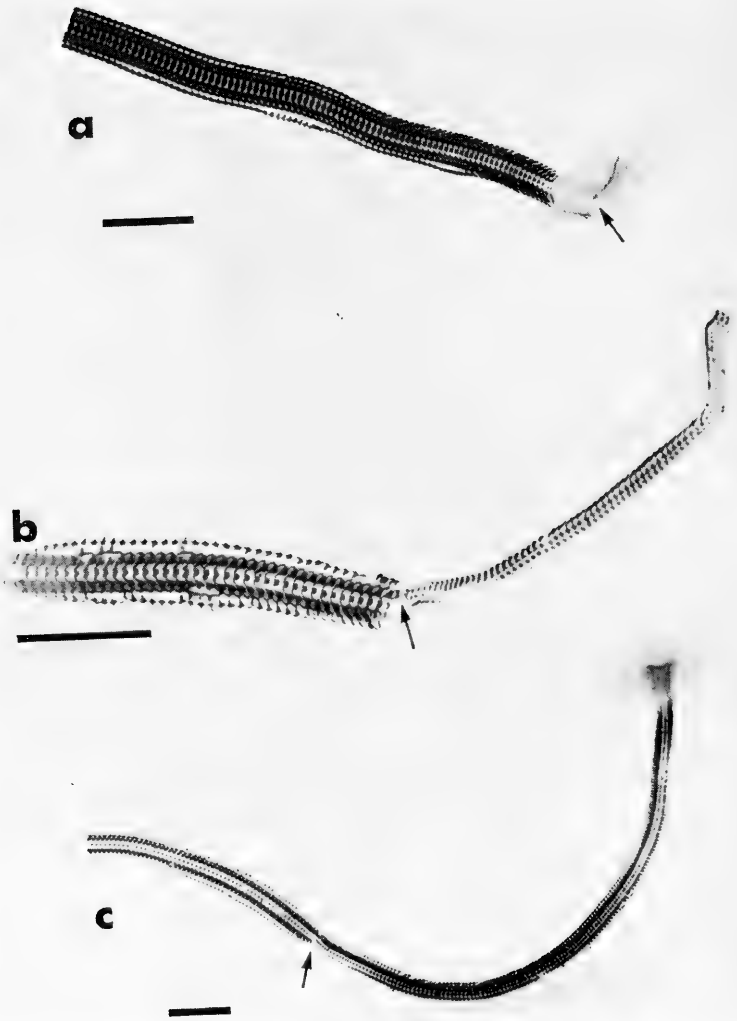

FIG. 5. Radulae of *Littorina littorea*. Only the posterior halves are shown. The arrows indicate the cauterized area; the part of the radula to the right of the arrows was formed after the operation:

a. After 10 days.
b. After 35 days.
c. After 110 days.

The *Helix aspersa* radula is of the typical short and broad pulmonate type while that of the prosobranch *Littorina littorea* is very long and narrow. In spite of this difference, radula replacement in the latter species (Figs. 5 and 6) was found to be very similar to that in *Helix aspersa*. The experiments were carried out at 10° C $\pm$ 1° C with mature *Littorina* (shell height 2.5 - 3.0 cm). Animals were killed at 5 day intervals up to the 195th day after operation and their radulas examined. The average rate of secretion of the radular teeth in 43 ani-

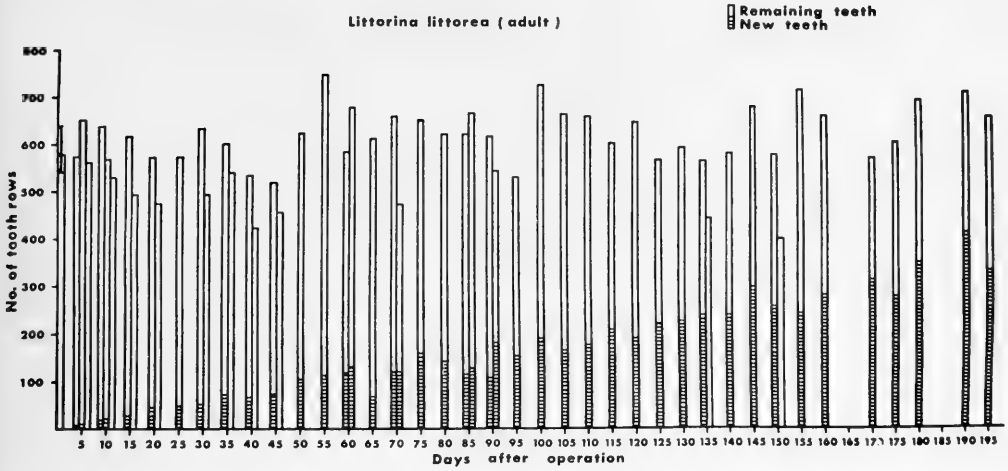

FIG. 6. Tooth replacement in adult *Littorina littorea* at 10° C. (constant). Each column represents the radula of 1 animal except for the control column (left) which is the average of 10 radulae (maximum and minimum is shown by the barred line). The shaded part of each column indicates the number of rows of teeth in the radula that were secreted after cautery and the unshaded part rows that were present at the time of the operation. The completely unshaded columns represent radulae whose odontoblasts had been totally cauterized by accident.

mals was 1.77 rows/day. Table 1 shows typical results obtained in *L. littorea*, at a temperature of 20° C, by the operative technique and those obtained by cold shocking. It can be seen that both sets of results are comparable, although those obtained by operation are somewhat lower, perhaps due to the longer recovery time. Experiments by both methods were carried out at different times of year and there seems to be no seasonal variation.

When experiments showed that temperature had a profound effect on the rate of secretion (see below) it was decided that, in order to compare the rate of secretion in different species, all experiments had to be carried out at the same temperature. 20° C was chosen as the most convenient temperature, although it was realized that it was low for *Achatina* and high for the marine forms. The radula was marked by cold shocking

TABLE 1. Summary of experiments to investigate the rate of tooth production in *Littorina littorea* at 20° C (constant). The animals were killed 20 days after the radula was marked

	Average shell size cm	Method of marking radula	No. of animals observed	Average rate rows/day (max.-min.)
	2.75 x 2.13	cold shock	40	4.97 (6.30-4.26)
	2.64 x 2.10	cold shock	30	5.46 (6.00-4.75)
	2.81 x 2.18	operation	18	4.92 (5.85-3.60)
	2.43 x 2.22	operation	4	4.90 (5.50-4.00)
Total	2.66 x 2.16	-	92	5.06 (6.30-3.60)

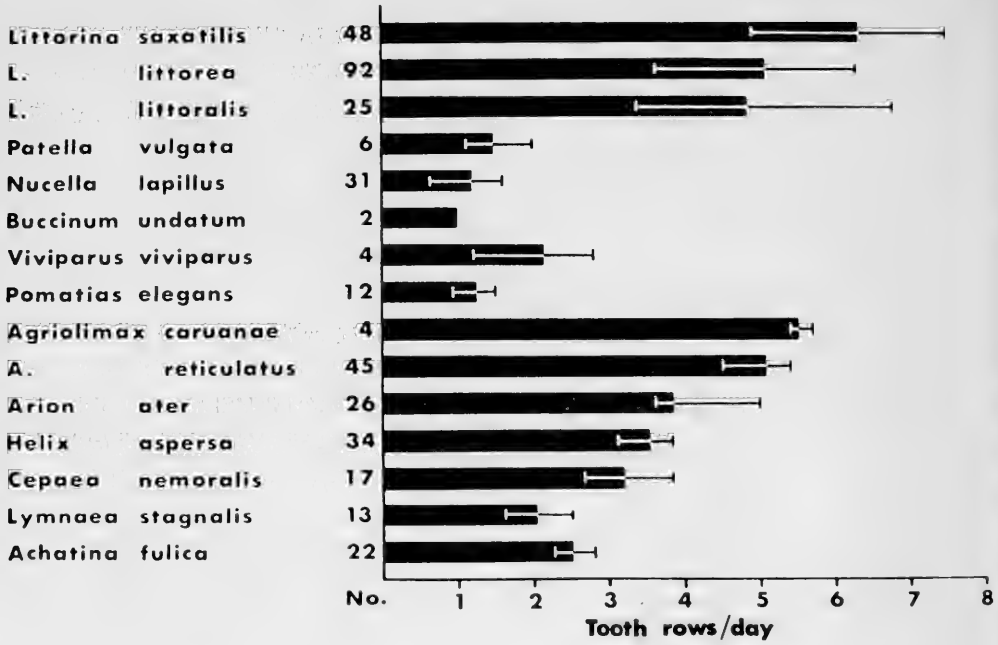

FIG. 7. Rates of radula secretion in 15 gastropods at 20° C (constant). The number of animals studied is shown opposite each species. Maximum and minimum values for tooth replacement rates are indicated by the barred lines. All animals were killed 20 days after the radula was marked.

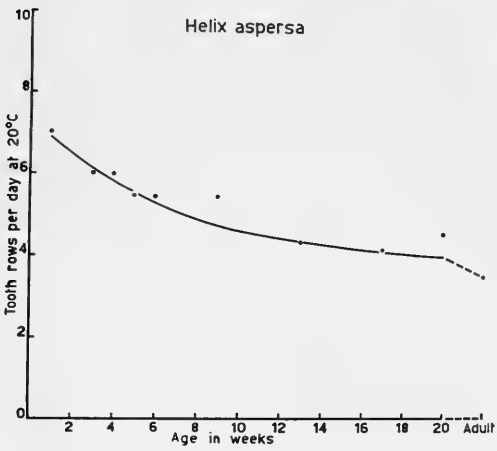

FIG. 8. Rates of radula replacement in *Helix aspersa* at different ages. Animals up to 2 months old were killed 5 days after the radula was marked, all other 10 days after.

in all cases, the animals were treated and kept in groups. They were killed after 20 days and the rate of secretion of new rows of teeth was determined. The results are given in Fig. 7.

The results for *Patella vulgata*, *Buccinum undatum*, *Viviparus viviparus* and *Agriolimax caruanae* are not as reliable as the others owing to the small numbers of animals (2-6) used. It can be seen that the fastest rates (5-6 rows/day) are those of *Agriolimax* and *Littorina* species, while the slowest (between 1 and 2 rows/day) are for *Patella*, *Thias* and *Buccinum*. As none of the 3 latter species survived well in our culture conditions and as it was found in *Helix* that moribund animals had a very slow radula replacement rate, it is possible that the latter rates are underestimates. It is regrettable that there are no reliable results for the carnivorous species.

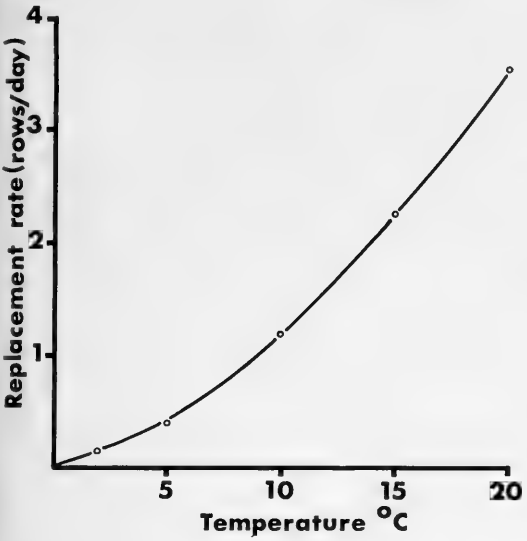

FIG. 9. Rates of radula replacement in adult *Helix aspersa* at different temperatures. All animals were killed 10 days after the radula was marked.

Effect of age on replacement

With the use of the cold shocking technique it is possible to mark the radulae of animals just after hatching. In this way the rate of replacement of the *Helix aspersa* radula at different ages was determined and the results are shown in Fig. 8. The rate of replacement was found to be very high in newly hatched animals (7.0 rows a day); this rate fell rapidly over the first few weeks and then more slowly to the adult level (3.6 rows a day at 20° C, constant). At the beginning of the experiment the range of shell sizes was quite small but considerable variation was evident by the end of the experiment.

Effect of size on replacement

It is well known that snails hatching from a clutch of eggs show very different growth rates, a fact that was confirmed here (see also above for *H. aspersa*). Both 10 and 12 month old *Achatina fulica*, each batch having hatched from clutches laid at about the same time, were sorted into 3 size groups and these were used to determine the possible influence of size on radula replacement. As can be seen from Table 2, the relation of rate of replacement to size was much closer than its relation to age. Thus, in both age groups, the larger the animals the slower was the rate of replacement.

Effects of temperature on replacement

The ambient temperature was found to affect the replacement rate very profoundly. The results for *Helix aspersa* and for the 3 species of *Littorina* at temperatures ranging from 0-20° C are summarized in Figs. 9 and 10. At 0° C the teeth were not being secreted

TABLE 2. Rate of tooth production in young *Achatina fulica* of the same age but of different sizes. The animals were killed 10 days after the radula had been marked

Age in months	Temperature °C	Nos. observed	Average shell size cm	Average rate rows/day (max.-min.)
10	20° - 25°	10	2.12 x 1.41	3.15 (3.40-2.90)
		10	3.06 x 2.36	2.31 (2.80-2.16)
		10	4.37 x 3.18	2.15 (2.60-2.01)
12	20° ± 0.5°	6	2.71 x 1.91	2.66 (2.80-2.55)
		5	3.48 x 2.48	2.44 (2.60-2.30)
		11	4.45 x 3.04	2.34 (2.60-2.25)

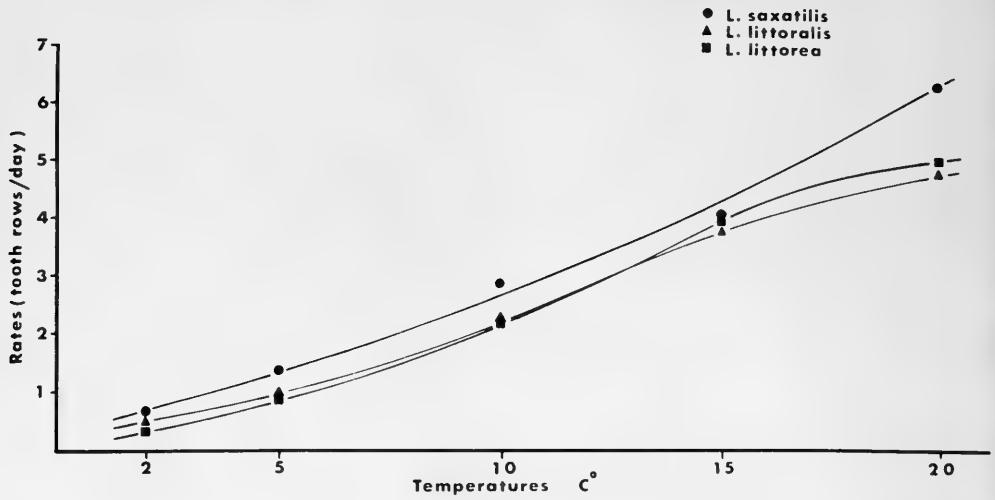

FIG. 10. Rates of radula replacement in adult *Littorina* spp. at different temperatures. All animals were killed 10 days after the radula was marked.

but above this temperature there was a steady increase in rate of replacement with increase in temperature. In *Littorina littorea* and *L. littoralis* it appeared that the rate was starting to decrease at the higher temperatures. The total number of rows of teeth and the size of the teeth in the radulae of the experimental animals were measured and no differences were found.

Replacement during dormancy

Dormant animals were disturbed as little as possible so as to avoid waking them. Aestivating *Helix aspersa* were characterized by thin mucous membranes that held their shells to the wall of the container, while in hibernating animals thick multilayered calcareous epiphragms were present.

The results of observations on 29 dormant snails kept at between 14° and 21° C are summarized in Table 3. The control animals were kept under similar temperature conditions. During aestivation teeth were added at a rate closely similar

TABLE 3. Rates of radular replacement in *Helix aspersa* during aestivation and hibernation. Control and aestivated animals were killed at 5 day intervals up to 100 days after marking the radula and hibernating animals at 10 day intervals up to 100 days

Conditions	Average shell size cm	No. of animals studied	Temperature °C	Average rate rows/day (max.-min.)
Control	2.86 x 2.90	22	10 - 20°	2.45 (2.10 - 2.95)
Aestivation	2.77 x 2.88	20	15 - 21°	2.41 (1.76 - 3.24)
Hibernation	2.72 x 2.91	9	14 - 17°	1.38 (1.14 - 1.66)

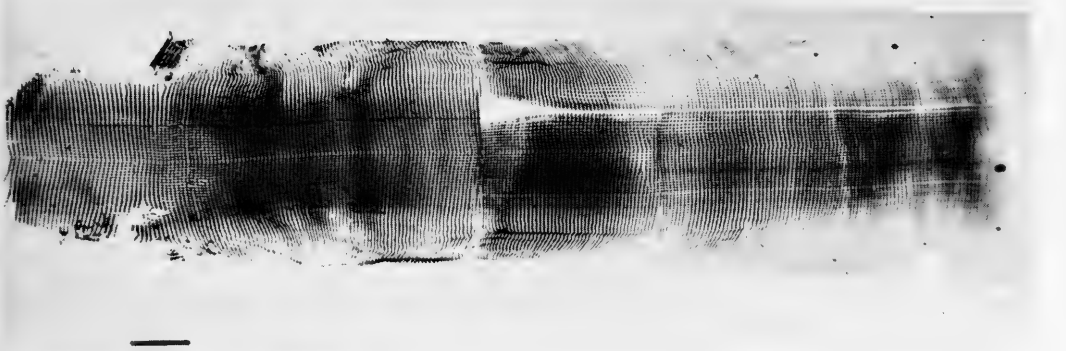

FIG. 11. Radula of hibernating *Helix aspersa* 100 days after operation; 269 rows are present, more than twice the normal number.

to that in control animals (2.41 against 2.45 rows/day). The old teeth were not, however, lost at an equivalent rate so that the radula became much longer. This increase in length was due to 2 factors. 1) While the oldest teeth at the very front of the radula detached from the underlying epithelium in the normal way, they remained connected by the uppermost layer of membrane. There was thus an accumulation of these old teeth and on dissection these were seen to be folded up in the buccal cavity. If the animals were allowed to wake up before being killed, they usually rasped the rims of their shells and these accumulated old teeth were then found as large chunks in the gut. 2) There was an increase in the actual length of the radula and its surrounding cellular layers, and, as the collostyle appeared to remain the same length, this led to a 'corrugation' of the radula.

During hibernation in the laboratory, radula replacement still occurred, but at a rate somewhat slower than in the controls (1.38 against 2.45 rows/day). Again there was an accumulation of old teeth and 'corrugation' (Fig. 11). In some cases, although animals were killed before waking, the old teeth had become detached; it is believed that this was due to occasional waking of the animals without protrusion from the shell. When animals had hibernated on the side of a glass container such movements were sometimes seen to occur.

Forward movement of radula after destruction of its posterior end

To observe the forward motion of the radula and the loss of teeth at its front end independently of the supply of new teeth from the rear, the hind end of the radula was removed in one of 2 ways: either by severe cautery, when the odontoblasts and the first few rows of teeth were destroyed, or by cutting off the posterior 30-40 rows of teeth. To determine the rate of loss of old teeth after such removal is much less accurate than to measure the number of new teeth secreted, because of the variation found in the total number of radula rows, a number that is not known at the beginning of the experiment. Results obtained for *Helix* and *Littorina littorea* are summarized in Table 4. It can be seen that the old teeth were lost at least as fast as new teeth are normally added in animals with complete radulae. The posterior part of the radula does not therefore appear to be necessary for the production of the forward movement

TABLE 4. Estimated rate of loss of old teeth from the anterior part of the radula of *Helix* and *Littorina* after removal of its posterior part

Species and method	No. of animals studied	Temperature °C	Estimated rate of tooth loss (rows/day)	Rate of tooth production in controls (rows/day)
Helix aspersa				
by cautery	3	10 - 20°	2.95	2.45
by excision	5	10 - 20°	2.24	2.45
Littorina littorea				
by cautery	10	10°	3.54	2.20
by excision	1	20°	9.45	5.06
	3	10°	4.42	2.20

TABLE 5. Radula replacement in auto-, homo- and heterografts of the radula in the haemocoel

Type of graft	Donor	Host	No. of animals	Temperature °C	Days after transplanting	Rate of replacement rows/day	
						observed	controls
Auto-	*Helix aspersa*		3	16-18°	10	2.17	2.80
	H. aspersa		4	20-22°	10	2.93	3.20
	Littorina littorea		1	10°	20	1.05	1.95
Homo-	*H. aspersa*	*H. aspersa*	2	16-23°	20	3.40	3.44
	H. aspersa	hibernating *H. aspersa*	1	14-17°	100	1.48	1.38
Hetero-	*Arion ater* (young)	*H. aspersa*	2	17-23°	6	1.63	2.10 (adult, 10°)
	A. ater (adult)	*H. aspersa*	1	17-23°	40	0.0	2.10 (adult, 10°)
	L. littorea (young)	*H. aspersa*	2	17-23°	10	0.0	6.30

of its front part. This finding supports the conclusions previously reached (Runham, 1963) from an histological and autoradiographic study of the radula gland. In that study it was suggested that the radula is carried forward by the forward motion of the epithelium attached anteriorly to its underside. In one *Helix*, kept for 120 days, only bare membrane remained. This animal was

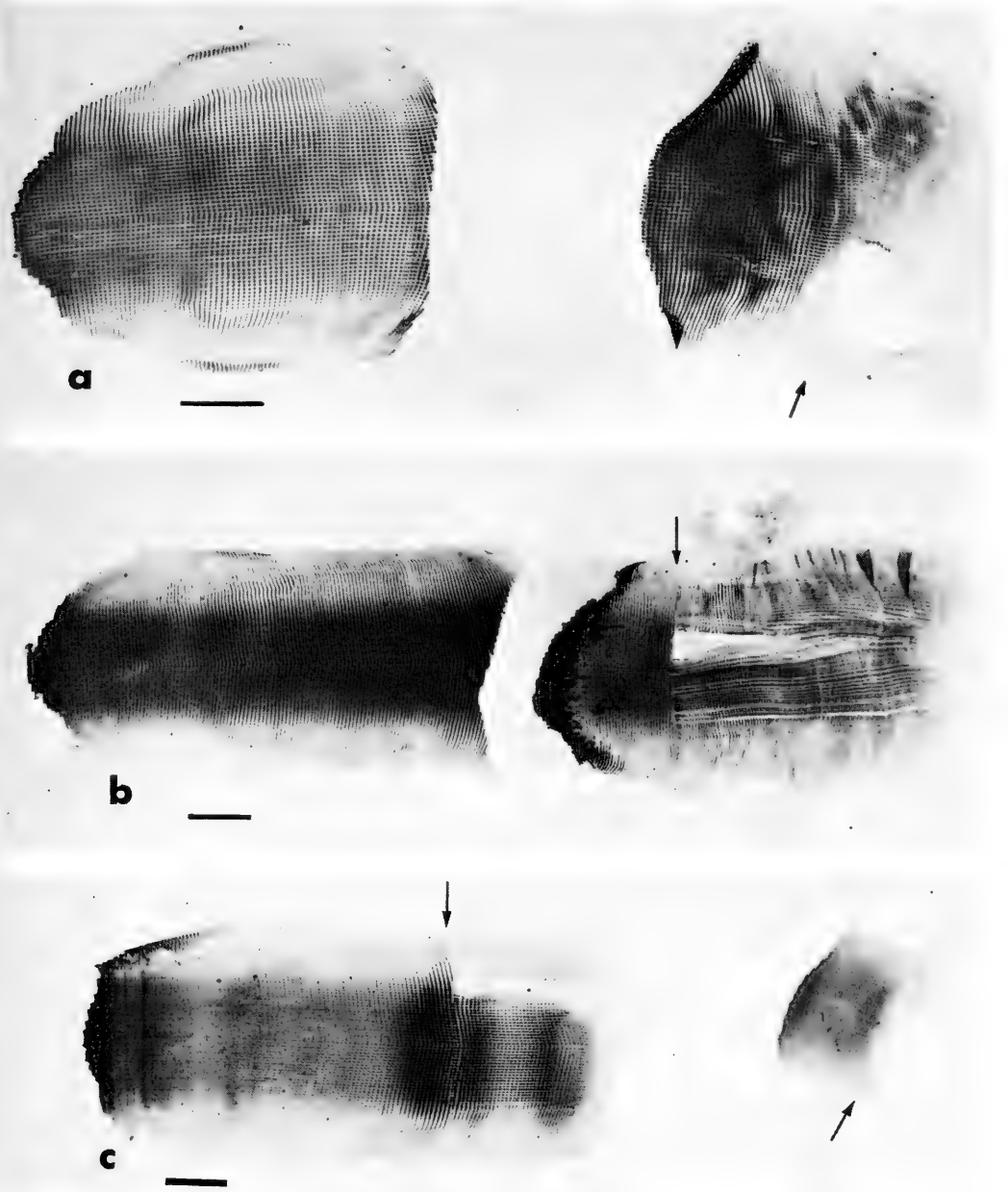

FIG. 12. Radula grafts in *Helix aspersa*. The pieces transplanted into the haemocoel are shown on the right side; note: in each case the rows secreted after transplanting are to the right of the arrows. On the left side are the "host" radulae (with posterior parts removed in a and b).

a. Autograft, 10 days after operation.
b. Homograft, 20 days after operation.
c. Heterograft (young *Arion ater* transplant), 10 days after operation.

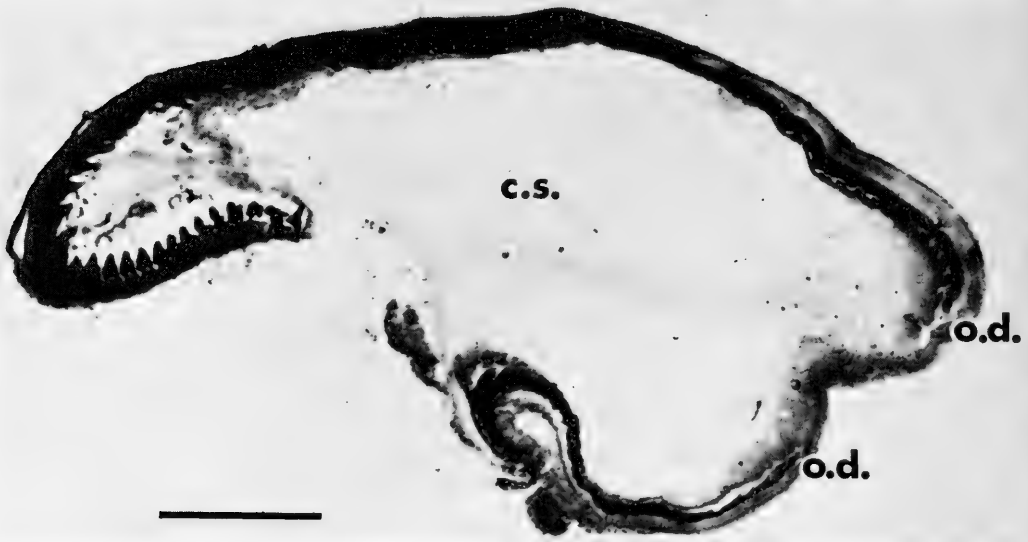

FIG. 13. 10-day *Helix aspersa* autotransplant. Longitudinal section. The radula has increased in length and at the anterior end (left) it has grown around the cut surface of the collostyle. c.s., collostyle; o.d., odontoblasts.

seen attempting to feed, but no sign of food or discarded radula teeth were found in the gut.

When *Helix* that had been operated on in this way were dissected, the radula gland appeared to have its normal shape, but this was due to regeneration of the collostyle.

Radular grafts into the haemocoel

The rates of replacement found in the grafts are shown in Table 5.

Replacement in the autografts proceeded at a similar rate to that found in the controls and was dependent upon temperature, just as in the whole radula. The presence of a graft in the haemocoel of an animal did not influence the rate of tooth loss from the remaining anterior part of its radula, in autografts, nor the rate of replacement of the radula in homografts. In heterografts, the rate of replacement was lower than normal when the grafts were from young and not too distantly related animals, e.g. when young *Arion ater* radulae were transplanted into *Helix*. However, no new teeth were formed with grafts from similar but older animals, or with more distantly related animals (e.g. *Littorina* into *Helix*). Whereas in the auto- and homografts there was very little if any breakdown of the graft, there was breakdown in heterografts at the cut end, in cases where replacement was found (Figs. 12a, b, c), or considerable destruction at both ends when no tooth production had occurred. The graft usually became curved, adopting an L- or a U-shape in longitudinal section (Fig. 13) and it appeared that the collostyle had remained the same length while the radula and the cellular sheath had increased in length.

DISCUSSION

Continuous replacement of the radula was found to occur in all the prosobranch and pulmonate species investigated. It is therefore likely that this phenomenon occurs throughout these groups. Although it was not possible to carry out studies on opisthobranchs, cephalopods, or scaphopods, it would appear likely from the structure of their radular gland, that replacement is found in these groups too.

Whenever duplicate experiments were carried out on groups of adult snails of the same species the replacement rates were usually similar. It appears therefore that the radula replacement rates are fairly constant in adult snails. As the growth rate of these adults is very low, it is likely that any changes in replacement rate of the radula associated with growth would be so small as to escape detection by the methods used.

The radula replacement rates in adults of different species varied between 1 and 6.4 rows/day, although the lowest rates are probably unreliable. The estimates by Sterki (1893) that the radulae of *Limax campestris* and *Polygyra thyroides* were changed entirely 8 and 16-18 times respectively during the life of the animal, are very probably, therefore, underestimates. It would appear that the most active herbivores have the highest replacement rates (*Agriolimax* and *Littorina*) while the sluggish forms (*Pomatias*) and perhaps the carnivorous forms (*Nucella*, *Buccinum*) have a slow rate. The rate of replacement of the radula may therefore be adapted to the feeding activity of the animal, i.e. the more active feeding, the greater is the wear on the radula and the faster the replacement rate. Many more species will have to be investigated before this relationship can be established with certainty. Such a relationship would appear to be complicated by the great variations that are found in hardness of the teeth (Lowenstam, 1962). In this investigation, however, the replacement rates of the active herbivores *Littorina littorea* and *Agriolimax reticulatus* were found to be very similar, although the teeth of *Littorina* are much harder. As cold shocking was found to be such a simple method of marking the radula it is to be hoped that many more species will now be studied.

It was found in this and in previous work (Runham, 1962) that as new rows of teeth are added to the radula at the rear the old ones are lost in front. Normally the radula of the adult remains a more or less constant length. There would thus appear to be a dynamic equilibrium between production and loss of teeth. This equilibrium must involve several processes: secretion and shaping of new teeth, movement forwards and hardening of the new teeth, use of the teeth in feeding and detachment of the oldest teeth from the front of the radula. Replacement of the radula is thus a very complex and highly integrated process.

In a very detailed study of the radula of *Lymnaea limosa*, Hubendick (1945) was able to demonstrate conclusively that the length and width of the radula and the number of teeth were proportional to the size of the animal. It can also be seen from Hubendick's figures that the rate of increase in these radula characteristics was fastest in the smallest animals. As can be seen from Table 2, the rate of replacement in 1-year old *Achatina* is similarly closely related to size and appears to be independent of age. When very young animals are studied, however, it can be seen (Fig. 8) that in these there is a close correspondence between replacement rate and age.

The results obtained in this study indicate that replacement rate decreases with age in young animals, whereas in older animals, when there is greater variation in size due to differences in growth rate, it becomes proportional to shell size. Perlowagora-Szumlewicz & von Brand (1958) have shown in *Australorbis* that metabolism is proportional to size rather than age, while other workers have also shown that young animals have a higher metabolic rate than older ones.

Temperature is a factor which strongly influences metabolic rate and much work has been carried out in this field. For every 10° C rise the rate increases 2-3 times (Q_{10} = 2-3). The results obtained for *Helix* and *Littorina* fall within this range (Figs. 9 and 10). As radula replacement involves at least 4 processes and no differences could be found either in the lengths of the radulae

or in the size of the teeth in animals kept at different temperatures, temperature must affect all the processes equally. It is possible that longer term experiments might show differences, but any differential effect is likely to be small.

From the results obtained for the effects of age, size, temperature and species differences, it would appear that radula replacement is closely related to the metabolic rate of the animal. Yet, the effects of hibernation and aestivation apparently refute this suggestion. During hibernation and aestivation the activity of gastropods is reduced to a minimum (Wells, 1944; Kilian, 1951); the heart almost or completely stops beating and respiration is at a very low level. This was confirmed in hibernating young *Helix*, in which it was possible to see through the transparent shell that the heart had completely stopped beating. In spite of this, however, new radula was secreted at almost the same rate as in control animals. A normal rate of secretion was also found in a homograft in the haemocoel of a hibernating animal (Table 4). While this secretion was not apparently affected by the metabolic rate of the animal, the forward movement of the radula was. Some movement forward did occur, as evidenced by the accumulation of old teeth in the buccal cavity, but at a rate slower than secretion of new radula and formation of new epithelia, resulting in 'corrugations'. Detachment of the old teeth apparently occurred normally, but in many cases the teeth did not become separated. Though chemical disintegration of the lower membrane had occurred, the mechanical forces, involved in active rasping, appear to be needed to break the thin upper membrane holding the teeth together. The differential effect produced by these dormant states clearly emphasizes the separate mechanisms involved in replacement.

Although separate mechanisms are involved, they normally proceed at very similar rates. This was shown very clearly by the grafts, and in experiments in which the posterior part of the radula had been destroyed. In these cases, production of new radula at the posterior end after cutting off and grafting, and the movement forwards of the radula in its anterior part occurred at the same rate, even though these parts were separated. The forward movement thus appears to be completely independent of production of new radula. Because the grafts possibly included some of the anterior attached epithelium that is involved in the forward carriage of the radula, it cannot be completely ruled out that forward movement may influence secretion. Because there was little difference between the rate of replacement found in auto- and homografts, it is concluded that, if there is any adverse homograft reaction, it does not affect radula production. This absence of a response agrees with results obtained by other workers with auto- and homografts of the reproductive tract (Laviolette, 1954) and of various tissues (Tripp, 1963). There was a very definite heterograft response, as shown by the reduced rate of replacement and the breakdown of the transplant. An adverse heterograft reaction was reported by Tripp, but Laviolette obtained successful heterografts. This last author, however, transplanted immature reproductive tracts and gonads to other closely related slugs. The heterograft reaction described in this paper was also weakest with young closely related animals. It is interesting that Mattinson (1965) reports a sequential development of antigens to occur throughout life in some slugs. The presence of radula grafts in the haemocoel never affected normal replacement of the host's radula nor its forward movement.

It is hoped that, as the techniques used in these studies are relatively simple, they will be extended to a much wider range of molluscs from different groups and habitats.

ACKNOWLEDGEMENTS

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RÉSUMÉ

ETUDE SUR LE REMPLACEMENT DE LA RADULA DE GASTROPODES

K. Isarankura et N. W. Runham

Les radula ont été marquées à leur extrémité postérieure par: cautérisation, injection de chlorure de magnesium, injection de colchicine, traitement par le froid. Par la suite, le déplacement vers l'avant de la partie marquée permet de mettre en évidence et de mesurer le remplacement radulaire. Les nouvelles techniques d'injection de chlorure de magnesium et de colchicine permettent des marquages de radula sans longue manipulation et le traitement par le froid permet de marquer la radula de petites espèces et de jeunes animaux chez lesquels on ne peut opérer, ni faire d'injections à cause de leur faible taille. Les diverses méthodes de marquage utilisées fournissent des résultats comparables. On a trouvé un remplacement continu chez tous les Prosobranches et Pulmonés examinés: *Littorina saxatilis*, *L. littorea*, *L. littoralis*, *Patella vulgata*, *Thais lapillus*, *Buccinum undatum*, *Viviparus viviparus*, *Pomatias elegans*, *Agriolimax caruanae*, *A. reticulatus*, *Arion ater*, *Helix aspersa*, *Cepaea nemoralis*, *Lymnaea stagnalis*, *Achatina fulica*.

Il y a un très rapide remplacement de la radula chez les individus récemment éclos, et ensuite une sérieuse diminution du taux de remplacement. Chez les plus vieux individus, ce taux varie avec la taille de la coquille, il est d'autant plus faible que la coquille est grande. Le taux de remplacement est proportionnel à la température.

Quand la partie postérieure de la radula est détruite, l'élimination des vieilles dents à l'extrémité antérieure se fait à un taux semblable à celui d'une production normale de la radula.

Pendant l'estivation, la vitesse de formation de la nouvelle radula est la même que chez les témoins, pendant l'hibernation elle est parfois plus lente. Cependant, le déplacement vers l'avant est plus lent que normalement, cela résulte du plissement de la radula sur le collostyle. Les vieilles dents ne peuvent pas se séparer, mais s'accumulent à l'extrémité antérieure de la radula.

Le taux de sécrétion de nouvelles dents, pour les auto-et homogreffes de la partie postérieure de la radula dans l'hemocoele, est semblable à celui de la radula complète; pour les hétérogreffes entre animaux jeunes et proches parents, le remplacement est plus lent que la normale, pour les hétérogreffes entre animaux agés ou de parenté éloignée, aucun remplacement n'a lieu.

RESUMEN

ESTUDIOS SOBRE LA REPOSICION DE DIENTES EN LA RADULA DE LOS GASTROPODOS

K. Isarankura y N. W. Runham

Se usaron los siguientes métodos para marcar las rádulas: Cauterio, inyección de cloruro de magnesio, y refrigeración. Las rádulas se marcaron en la parte posterior, así el movimiento hacia adelante de la parte marcada se usó para determinar la ocurrencia y la rapidez del reemplazo radular. Las técnicas nuevas del cloruro de magnesio y la inyección de colquicina hace que el marcado se opere sin mucha demora, y la refrigeración o "golpe de frío" permitió el marcado de ejemplares pequeños y jóvenes que no se podrian operar ni inyectar a causa de su mínimo tamaño. Los diferentes métodos dieron resultados comparables. Reposición contínua fué comprobada en todos los prosobranquios y pulmonados examinados: *Littorina saxatilis*, *L. littorea*, *L. littoralis*, *Patella vulgata*, *Thais lapillus*, *Buccinum undatum*, *Viviparus viviparus*, *Pomatias elegans*, *Agriolimax caruanae*, *A. reticulatus*, *Arion ater*, *Helix aspersa*, *Cepaea nemoralis*, *Lymnaea stagnalis*, *Achatina fulica*.

En individuos recien nacidos hay un reemplazamiento radular muy rápido, seguido

de una disminución constante en la velocidad. En individuos viejos la rapidez varía con el tamaño de la concha, siendo más lenta en los de mayor tamaño. La rapidez es también proporcional a la temperatura.

Cuando la parte posterior de la rádula de destruye, la pérdida de dientes viejos en su extremo anterior ocurre a una rapidez similar a aquella de la producción normal de la rádula.

Durante la estivación la velocidad de la formación de nueva rádula fue la misma que en los controles, pero algo más lenta durante la hibernación. El movimiento hacia adelante fué, sin embargo, más lento que el normal resultando en la corrugación de la rádula sobre el colostilo. Los dientes viejos no se separaron sino que se acumularon al frente de la rádula.

La secreción de dientes nuevos, en auto y homotransplantes de la parte posterior de la rádula dentro del homocelo, fué de una velocidad similar a la del total de la rádula; en heterotransplantes entre animales jovenes relacionados, el reemplazo fue más lento que el normal, y entre aquellos de animales viejos o menos relacionados no ocurrió reemplazo.

АБСТРАКТ

ИЗУЧЕНИЕ СМЕНЫ РАДУЛЫ У ГАСТРОПОД

К. ИЗЕРАНКУРА и Н. РЕНХЕМ

Радулы моллюсков были помечены различными способами: прижиганием, ин'екцией хлористой магнезии, колхицина или холодовым шоком. По продвижению вперед отмеченных мест определялись наличие смены радулы и ее скорости. Применение новой техники - ин'екции хлористой магнезии и колхицина сдалало возможным пометку радулы без длительной операции, а холодовый шок дал возможность метить радулу мелких форм и молоди, которые были слишком мелки для применения других операций. Примененные различные методы дали сравнимые результаты. У всех исследованных Prosobranchia и Pulmonata была найдена непрерывная смена радулы: *Littorina saxatilis, Littorina littorea, Littorina littiralis, Patella vulgata, Thais lapillus, Buccinum undatum, Viviparus viviparus, Pomatias elegans, Agriolimax caruanae, Agriolimax reticulatus, Arion ater, Helix aspersa, Cepaea nemoralis, Lymnaea stagnalis, Achatina fulica.* Очень большая скорость смены радулы наблюдалась у только что отродившейся молоди, сопровождавшаяся затем устойчивым замедлением этой скорости. У более старших возрастов скорость смены изменялась с изменением размеров раковины, будучи более медленной у более крупных экземпляров. Скорость смены радулы также пропорциональна температуре.

Во время эстивации (летнего периода покоя) скорость образования новой радулы была такой же, как в контроле, а во время зимнего покоя - несколько замедленной. Продвижение радулы вперед было, однако медленнее нормального, в результате чего происходило сморщивание радулы над колостилем. Старые зубцы не отделялись, а накапливались в передней части радулы.

Скорость образования новых зубцов при ауто и гетеропересадках задней части радулы в гемоцёль, была такой же, как и во всей радуле: пересадки между молодью родственных особей смена радулы была более медленной, чем нормально, а при пересадках, произведенных между старыми или менее близко-родственными особями, смены ее не происходило вообще.

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STUDIES ON THE MATURATION OF THE REPRODUCTIVE SYSTEM OF *AGRIOLIMAX RETICULATUS* (PULMONATA: LIMACIDAE)

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ABSTRACT

Agriolimax reticulatus Müller were collected in North Wales over a period of 18 months. Breeding, at a maximum in spring and autumn, occurred throughout the year. The reproductive tract of the slugs was dissected and its parts were separated, weighed and sectioned. The maturation stage of the hermaphrodite gland was not closely related to the weight of the animal. The weight of this gland increased to a maximum at the "spermatid stage" and then decreased. Sperm appeared in the hermaphrodite duct and spermatheca at the end of that stage, and were then present until the post-reproductive stage. The maturation of the albumen gland and common duct was closely related to that of the hermaphrodite gland. The glands on the sarcobellum mature at the "spermatozoon stage." These results appear to indicate that the stages in the maturation of the reproductive system are related to the growth phases of the animal, and that both physiological and environmental factors may control this maturation.

INTRODUCTION

The biology of the grey field slug *Agriolimas reticulatus* has been well studied (Quick, 1958; Frömming, 1954; Bett, 1960; Arias & Crowell, 1963; South, 1965). Details of the changes in the reproductive system associated with the breeding cycle are, however, not available for this species. In contrast, detailed descriptions are available for *Arion ater*, reared in the laboratory (Lūsis, 1961) and collected from the field (Smith, 1966), and for the American slug *Philomycus carolinianus* (Kugler, 1965).

As a preliminary to a study of the factors which control the breeding cycle in *Agriolimax reticulatus* a detailed study of the reproductive system was undertaken and is reported below.

MATERIALS AND METHODS

The 2 species *Agriolimax reticulatus* and *A. caruanae* are common in Caernarvonshire and Anglesey, North Wales, particularly on cultivated land. Although these 2 species occur together, they are readily distinguishable by external features.

Slugs were collected after nightfall at weekly intervals, throughout 1965 and thereafter monthly until the end of June, 1966. An area of rough ground at the base of a drystone wall in the college grounds was visited each time. Each sample consisted of the first 10 *Agriolimax reticulatus* discovered. The samples had to be restricted to this size because of the subsequent detailed investigation.

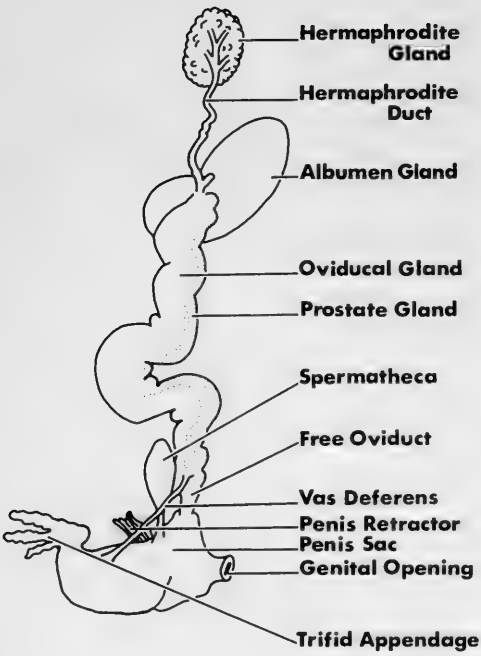

FIG. 1. Diagram of the reproductive tract of *Agriolimax reticulatus* (from Bayne, 1966).

The animals were kept overnight in a closed container together with damp moss or grass. Next morning they were freed from any attached soil by gentle rolling on filter paper and then weighed as quickly as possible to the nearest milligram on a torsion balance.

'Susa' fixative was injected into the slugs to kill them and then the reproductive system was dissected out. The genital tract was subdivided into hermaphrodite gland, hermaphrodite duct, albumen gland, common duct plus free oviduct, spermatheca, and penis together with vas deferens (Fig. 1). These parts were left in fresh fixative for 6-12 hours, then passed through 2 changes of cellusolve for 24 hours each. The pieces were removed one at a time, left for a very short time on filter paper to remove excess moisture and then weighed on a semi-micro balance to the nearest 0.01 mg. The weighings were reproducible, but as no attempt was made to determine the effect of the processing on the weight of the tissue the values obtained were only suitable for comparative purposes. The weight of the whole tract was obtained by summing the weights of its constituent parts.

After weighing, the pieces of reproductive tract were returned to cellusolve and then embedded in ester wax. Sections were cut at 5μ and stained in Azan triple stain.

Of the 706 slugs collected and weighed, 429 were dissected and sectioned while 18 were so small that they were sectioned whole. From the results obtained the means were determined and the fiducial (confidence) limits of these means calculated at the 95% confidence level (Bailey, 1959).

RESULTS

Breeding Season

Since very small animals were collected throughout the year (Fig. 2) it is likely that breeding occurs at all seasons. However, from the numbers of small animals there appeared to be 2 main breeding periods, one in spring and the other in autumn. These findings agree with those obtained by workers in other areas of this country (Bett, 1960).

Reproductive Tract

As with other pulmonates the structure of the reproductive tract is very complicated (Fig. 1) and reflects the complexity of its function. From the histogram (Fig. 3) showing the relation between the weight of the body and of the reproductive tract, it can be seen that there is initially a very slow increase in tract weight followed by a rapid growth phase and then by a further period of slow growth. When the parts of the tract were weighed separately (Fig. 4) considerable differences were found in the types of growth curve. These will be interpreted after the histological descriptions.

Eighteen animals smaller than 80 mg could not be considered here, as it was

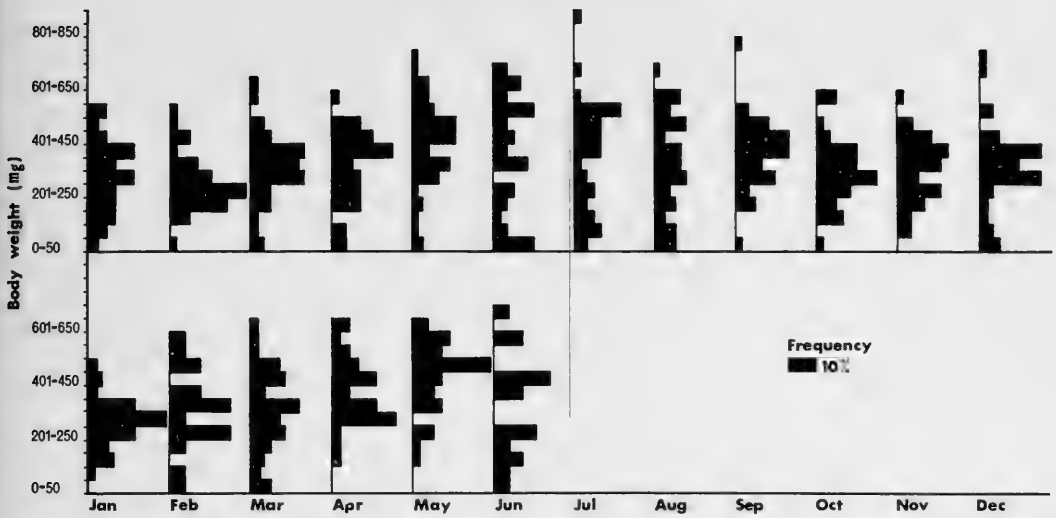

FIG. 2. Percentage frequency of weight groups per month of *Agriolimax reticulatus* collected during 1965 (top) and 1966 (bottom).

difficult to dissect out the reproductive tract cleanly and because of the large error involved in weighing such small organs. The juveniles were, however, sectioned whole in order to study the histology of their genital tracts. These were always continuous, as in older animals, even as early as 4 days after hatching in the laboratory. These findings do not tally with Richter's (1935), who reports that, until 10 days after hatching, the hermaphrodite gland of *Agriolimax* was separate from the rest of the tract.

Hermaphrodite Gland

The cytology of the cells in the hermaphrodite gland has been studied by Gatenby (1918). Richter (1935) carried out a very thorough analysis of the changes in structure of the hermaphrodite gland during maturation. The maturation changes in the gland take place in a continuous orderly sequence but in order to analyse these changes it was convenient to subdivide the development of the gland into a number of stages, i.e. the undifferentiated, spermatocyte, spermatid, early and late spermatozoon, early and late oocyte and post-reproductive stages. While these stages are quite readily distinguished, they do grade into each other. The morphological criteria on which they are based are as follows.

A. Undifferentiated Stage

The gland could not be weighed accurately at this stage. In sections it was seen to be a simple structure containing a solid mass of small undifferentiated cells.

B. Spermatocyte Stage

It is possible to weigh the gland accurately at this stage. Acini have budded out from the main mass of the gland, but it is still filled with a solid mass of cells. Within this mass can now be recognised some or all of the following cell types: spermatogonia (small nuclei with granular chromatin and 1 or occasionally 2 nucleoli), primary spermatocytes (large nuclei with a chromatin network), secondary spermatocytes (small nuclei with a fairly large amount of cytoplasm), oocytes (large nuclei with a very large nucleolus, and a large amount of cytoplasm, often containing yellow staining granules) and nurse cells (very large

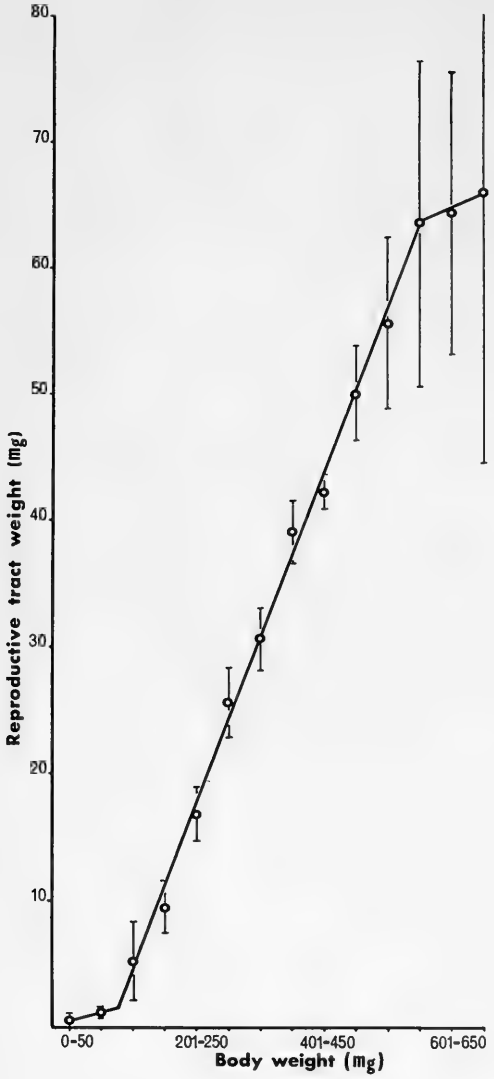

FIG. 3. The average weight of the reproductive tract compared to the body weight. The fiducial limits of the means (95% significance) are shown by the barred lines.

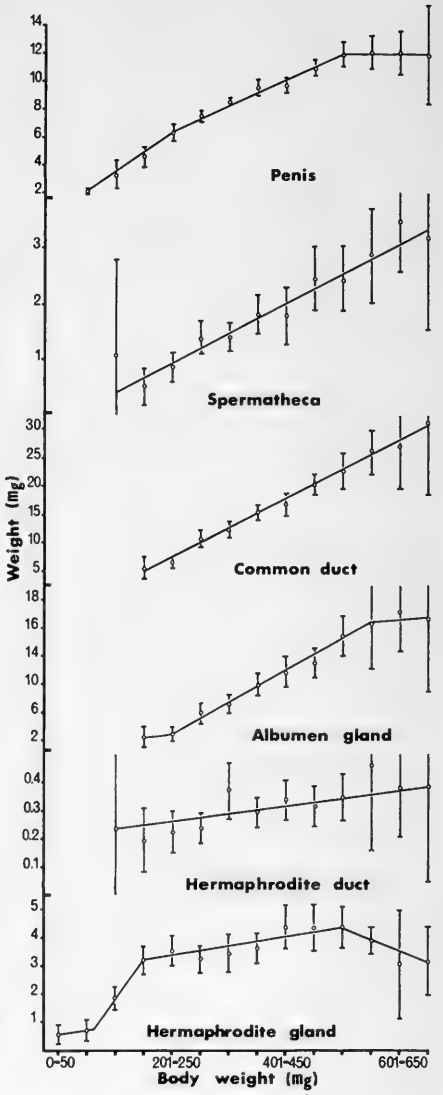

FIG. 4. The average weights of the various organs of the reproductive tract compared to the body weight. The fiducial limits of the means (95% significance) are shown (barred lines).

nuclei with very finely granular chromatin). The latter 2 cell types are usually found attached to the acinar wall.

C. Spermatid Stage

Ducts have appeared in the gland but the acini are still fairly solid. There are now many secondary spermatocytes and spermatids (dark blue to pink staining, evenly stained dense nuclei and sperm tails surrounded by cytoplasm). These 2 cell types tend to be found in groups most frequently towards the centre of the acini.

D. Early Spermatozoon Stage

A lumen is now present in the acinus although it is largely filled with sperm tails. The sperm have characteristically shaped, deep red staining heads and definitive sperm tails. A thick layer of cells in earlier spermatogenesis stages still lines the acinar walls. The sperm tend to be associated in groups related to a nurse cell.

E. Late Spermatozoon Stage

There is a very clear lumen in each acinus and many unattached sperm may be present there. The layer of cells in the earlier spermatogenesis stages is much thinner.

F. Early Oöcyte Stage

The sperm are still present in large numbers but many of the oöcytes are now very large. The oöcytes are covered by a thin layer of cells which forms a follicle. Some very small oöcytes are present, particularly at the narrow neck of each acinus where it opens into a collecting duct. These are possibly the most recently differentiated oöcytes, said by Richter (1935) to be produced in these areas and then to migrate into the base of the acinus.

G. Late Oöcyte Stage

Although still present, the sperm are reduced in number. At least some oöcytes have detached from the wall and are free in the lumen.

H. Post-reproductive Stage

A cuboidal epithelium, not found at any other stage, can be seen to cover at least part of the acinar wall. There is great variation in the numbers of sperm and ova remaining within the acinus.

Little doubt exists as to the sequence of the earlier stages, but some variation in the order of appearance of the "late spermatozoon" and the 2 "oöcyte" stages seems possible. Some slides of late spermatozoon stages revealed an apparent loss of most of their oöcytes. Similarly, at the late oöcyte stage a considerable number of sperm, spermatids, and spermatocytes were still present in some animals, while in others there were few. It is not known whether such variance reflects a variation in the relative amount of sperm and ova developed, or whether egg laying has taken place.

Some slugs, which had parasites in their hermaphrodite gland, will be considered separately (p. 15). It is also possible that a few animals were so very lightly infested that the parasites escaped detection.

When the stages of the hermaphrodite gland and the weights of the animals are compared (Fig. 5) it can be seen that, although there is a pronounced relationship between them, there is considerable variation. Thus late oöcyte stages (G) were found in animals weighing as little as 100 mg while spermatid stages (C) were still to be found in those as large as 350 mg. The relation of the stages of the gland to the time of year was also investigated but no correlation could be found.

From the weight of the gland it can be seen that there is an increase in weight up to the early spermatozoon stage (D, Fig. 6) which is associated with the phase of massive spermatogenesis. As the sperm was voided from the gland, particularly in the oöcyte and post-reproductive stages, so the weight decreased, and this loss was reflected histologically by the shrunken acini.

The uneven growth curve for the hermaphrodite gland, shown in Fig. 4, can now be interpreted in the light of the above findings. The initial slow growth is due to the prevalence of "differentiation" and "spermatocyte" stages in the lower weight groups. Spermatogenesis results in the phase of rapid growth while the decrease in weight found in the largest animals is due to the preponderance of the oöcyte and post-reproductive stages.

The Hermaphrodite Duct

The acini open into small collecting ducts which lead into the hermaphrodite

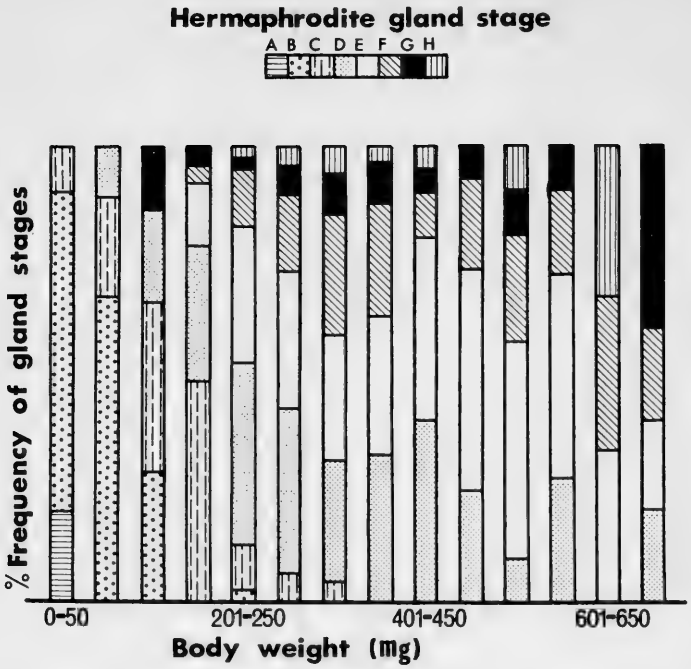

FIG. 5. Percentage frequency of the hermaphrodite gland stages in the different body weight groups. These stages are: A, undifferentiated; B, spermatocyte; C, spermatid; D, early spermatozoon; E, late spermatozoon; F, early oöcyte; G, late oöcyte; H, post-reproductive.

duct. It is a simple tube lined by a ciliated endothelium. Sperm appear in its lumen in the early spermatozoon stage and it is filled with them in subsequent stages, except for the post-reproductive stage. In the latter stage there was some variation: about half of the ducts examined were full, while the others were empty. Due to its small size the hermaphrodite duct was not dissected from the remainder of the reproductive tract in 63 of the smallest animals so that very few of the empty ducts were weighed. As can be seen from Fig. 4 there was considerable variation in the weight of the duct and no significant correlation with the animals' weight could be found.

The Albumen Gland

The hermaphrodite duct opens into the lumen of this gland near the point where it discharges into the upper end of the common duct. The maturation of the albumen gland has been subdivided, for convenience, into stages, but as with the hermaphrodite gland, its development is continuous.

A. Undifferentiated Stage

In very young animals the albumen gland is present as a small hollow diverticulum at the junction of the hermaphrodite duct and the common duct. It enlarges and its walls become folded.

B. Differentiation Stage

Mitoses are very frequent in the epithelium at this stage and tubules extend out from the walls of the diverticulum to form acini. The lining epithelium is columnar.

C. Maturation Stage

The acini are well differentiated and so are the collecting ducts. The cells

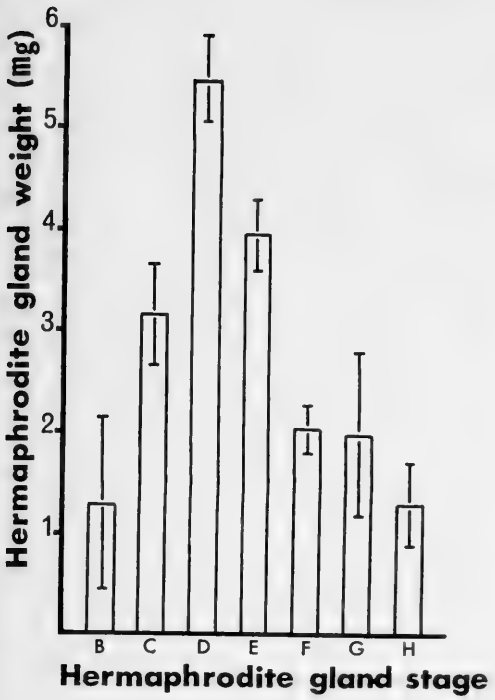

FIG. 6. The average weights of the hermaphrodite gland at its various developmental stages (the same as in Fig. 5). The fiducial limits of the means (95% significance) are shown by the barred lines.

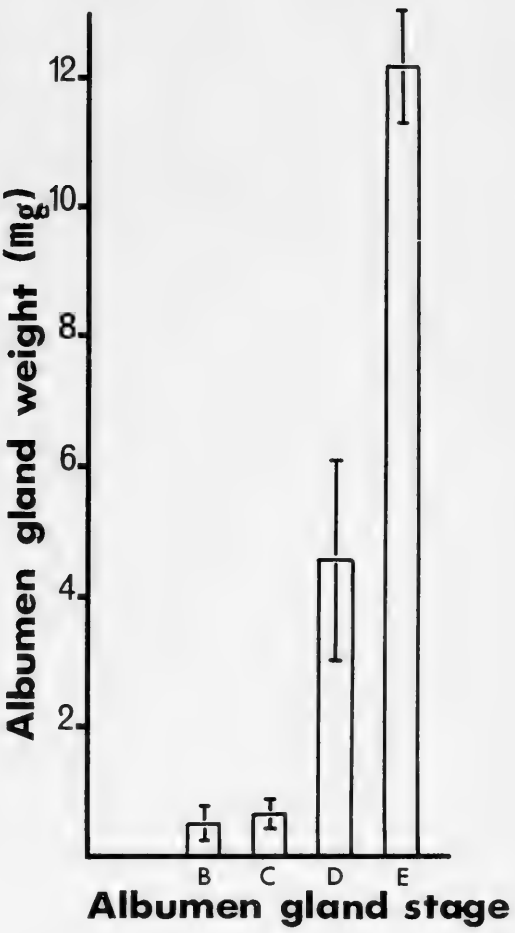

FIG. 7. The average weights of the albumen gland at its various developmental stages. The fiducial limits of the means (95% significance) are shown by the barred lines. The albumen gland stages (for this figure and for Fig. 8) are: A, undifferentiated; B, differentiation; C, maturation; D, accumulation; E, secretion.

have become more cuboidal but secretory granules are absent.

D. Accumulation Stage

Secretion first appears in the cells as small, red staining granules, but as more granules accumulate, they stain a light blue. The cells gradually fill up and become distended with the secretion.

E. Secretion Stage

The secretion passes from the cells into the lumen of the acini and thence into the collecting ducts. This secretion appears to be released by a breakdown of the apical regions of the cells.

The changes in complexity and size of the albumen gland are clearly reflected in its weight (Fig. 7). Thus the 3 earliest stages are associated with an increase in complexity but only a slight increase in size. As the secretion accumulates there is an increase in weight so that, when secretion appears in the lumen, the gland is very large. A similar relation is also shown by a comparison of gland and body weights (Fig. 4). At its maximum size this gland constitutes a large part, up to 6%, of the body weight.

When the stages of the albumen gland

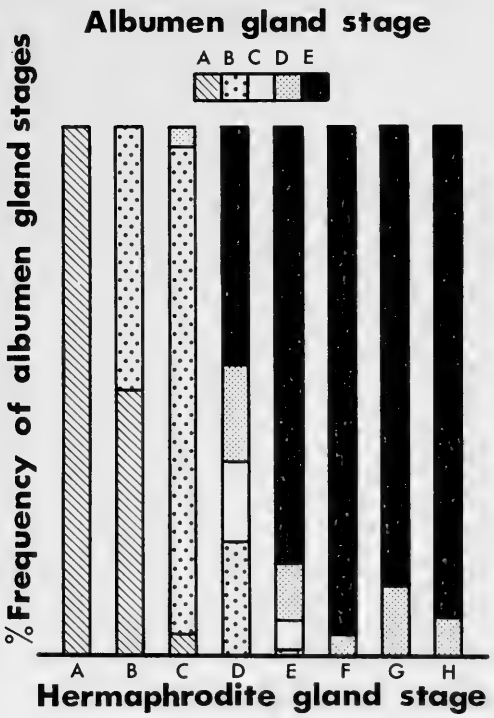

FIG. 8. Percentage frequency of the albumen gland stages at each hermaphrodite gland stage. The hermaphrodite gland stages (A-H) are the same as in Fig. 5, and the albumen gland stages (A-E) as in Fig. 7.

are compared with those of the hermaphrodite gland it can be seen that there is a very close relation between them (Fig. 8). Very few albumen glands were found at the maturation or accumulation stages, so it is possible that these stages are only transitory.

The Common Duct and Free Oviduct

The lumen of the common duct is partially subdivided by lateral folds into male and female ducts. The glands opening into the male duct constitute the prostate gland and those opening into the female duct the oviducal gland. At the lower end of the common duct the female duct continues as the free oviduct while the male duct continues as a completely separate vas deferens. Some details of the structure of this part of the tract and the cytology of the gland cells have been presented by Filhol (1938). Due to the presence and distribution of different glands and their varying rates of differentiation, determination of the changes occurring in the common duct during maturation was extremely difficult. Four stages could, however, be distinguished.

A. Differentiation Stage

Finger-like diverticula are present on the wall of the male side, being more frequent towards the distal part of the duct than in its proximal part. Many cell divisions are visible and the diverticula increase in number and length until the definitive prostate gland is formed. At this stage, however, the cells in the gland appear to lack cilia and secretory granules. The female part of the common duct remains undifferentiated. It is lined by a columnar epithelium and there is a dense underlying stroma in which later develop the oviducal gland cells.

B. Start of Male Secretion

The male duct is lined by a ciliated cuboidal epithelium which is interrupted by the openings of the branched diverticula. The cells of the diverticula are also ciliated. They now contain various types of secretion granules. Around the bases of the diverticula scattered strands of muscle can be found. At least 3 types of glandular cell can be recognised. (1) Situated mainly in the bases of the diverticula, in the upper part of the tract, are flask-shaped gland cells containing large yellow or blue staining granules. Some cells contained only one type of granule, the others a mixture of both. (2) Occurring mainly in the tips of the diverticula are similarly shaped cells containing very fine red staining spheroidal granules. This second type of cell was also reduced in number or absent from the lower part of the tract. (3) Concentrated in the lower part of the tract is a third type of cell in which the secretion, sometimes granular, but usually a large non-granular mass, stains blue. In the upper part of the tract, immediately

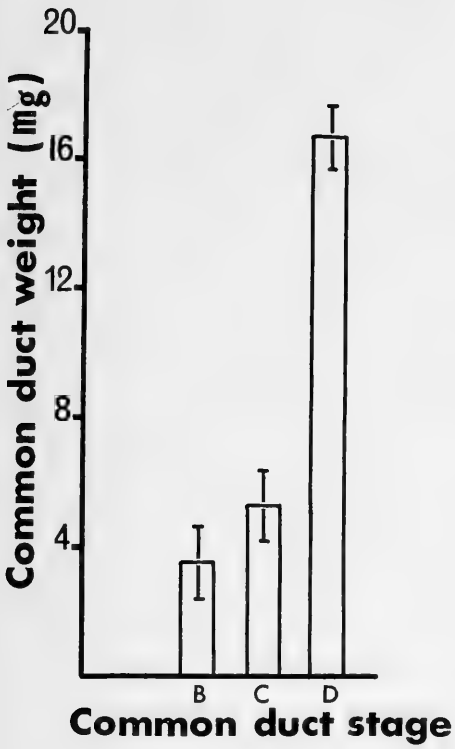

FIG. 9. The average weights of the common duct at its various developmental stages. The fiducial limits of the means (95% significance) are shown by the barred lines. The common duct stages (for this figure and for Fig. 8) are: A, differentiation; B, start of male secretion; C, start of female secretion; D, accumulation of female secretion.

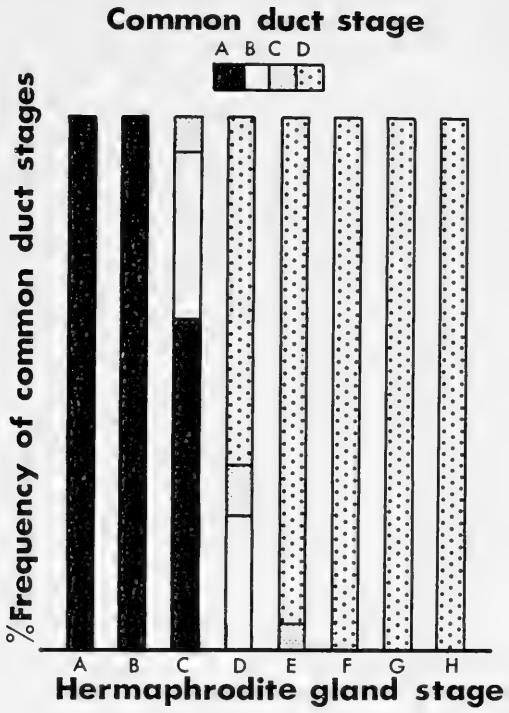

FIG. 10. Percentage frequency of common duct stages at each hermaphrodite gland stage. The hermaphrodite gland stages are the same as in Fig. 5, and the common duct stages as in Fig. 9.

underlying the epithelium of the male duct, there are some cells with a blue secretion which may be homologous with the third type described.

C. Start of Female Secretion

The cells underlying the epithelium of the female part of the common duct have differentiated into the long flask-shaped oviducal gland cells and a blue secretion has appeared in them. A small amount of this secretion is also seen in the lumen. There is a great reduction in the number of these glands towards the lower end of the tract. In contrast to the cuboidal epithelium in the male duct the ciliated epithelium in the female duct is columnar. As secretion accumulates in the cells it stains a lighter blue.

D. Accumulation of Female Secretion

The prostate gland cells are apparently as full of secretion as in the previous stage. The oviducal gland cells are now completely distended with secretion staining a very light blue. As a result of this distension, most of the ciliated epithelium becomes very thin. It is frequently interrupted by the large ducts. When an egg is found in the tract, it can be seen that the oviducal gland secretion forms the multilayered shell. Short strands of muscle are seen at all levels between the oviducal gland cells.

This great expansion of the female gland cells results in a very great increase in the total weight of the tract at

this stage (Fig. 9). As can be seen, however, from Fig. 4, there is a steady growth of the tract throughout the life of the animal. As noted for the albumen gland, a strong correlation with the stages of the hermaphrodite gland was similarly very noticeable for the common duct (Fig. 10). Thus, during the undifferentiated and spermatocyte stages of the hermaphrodite gland, the duct is in its differentiation stage. During the spermatid and early spermatozoon stages of the hermaphrodite gland, the male part of the common duct completes its maturation and the female part starts its maturation. The majority of the animals in the late spermatozoon stage and all those in the later stages have both parts of the genital tract mature.

The histology of the free oviduct did not appear to vary very much. It is a ciliated duct with a surrounding mass of flask cells opening into it.

Spermatheca

This is a simple sac-like organ lined by a ciliated columnar epithelium. There is considerable variation in its weight, but it appears to increase in size as the animal gets larger (Fig. 4). Sperm together with sperm mass material is present in its lumen from the spermatid stage onward. Thus, if it is correct that at copulation the partner's sperm is stored in it for at least a short time, then copulation must have occurred at that stage. In the late oocyte stage, fewer sperm are to be found and considerably fewer at the post-reproductive stage.

Penis and Vas Deferens

The penis is an eversible sac containing the distensible sarcobellum. Its structure is very complex. Few changes are visible during the maturation of the penis, and even in the youngest animals all the adult structures appear to be visible. It enlarges throughout most of the life of the animals (Fig. 4), and cell divisions are found up to the early spermatozoon stage. Gland cells are scarce except in the sarcobellum. In the latter, they are present at the spermatid stage, but secretion does not appear in the cells or in the lumen until the early spermatozoon stage. Over most of the sarcobellum these cells contain a blue secretion, but around its dorsal posterior border there is a band of cells whose secretion dissolved out during preparative treatment. Sperm are found in the lumen of the penis from the end of the early spermatozoon stage.

The trifid penial appendages are interesting structures. Until the early spermatozoon stage they are hollow, but then their epithelium becomes stratified and squamous, shedding cells into the lumen. In some areas the epithelium remains columnar but the cells appear necrotic and breakdown products appear in the lumen. A similar picture is also seen in the epithelium covering the rest of the penis. In some animals the appendages may be completely filled by sloughed cells and cell detritus. In others they may be full of a blue secretion apparently identical to that secreted by the cells of the lower end of the common duct. The breakdown of the epithelium is more frequent in the oöcyte and post-reproductive stages. It is possible that it may be associated with the decreased growth rate of the penis in the largest animals (Fig. 4).

The vas deferens is a simple ciliated and apparently non-glandular duct. In a few cases it contained sloughed cells.

Parasitised Animals

Animals found to contain parasites were excluded from the above study.

The most common parasite found was a protozoan which resembled *Tetrahymena limacis* (Arias & Crowell, 1963). This or a similar parasite has been observed, sometimes in large numbers, swimming in the perivitelline fluid of the eggs and occasionally entrapped in the egg shell (Bayne, in press). Other parasites found were nematodes, a larval trematode and another protozoan. As these parasites were observed in sections only, identification was not possible. In most cases

only a part of the hermaphrodite gland was disorganised so that its developmental stage could be identified from the remainder of the gland. In one animal only was the reproductive tract markedly retarded in its development: the hermaphrodite gland was in an oocyte stage, while the female glands in the tract had not completed their maturation (Stage C). This animal was not very heavily parasitised, but it was the only one in which there was any sign of parasitic castration.

DISCUSSION

The pulmonate reproductive tract is very complicated histologically (Lūsis, 1961; Smith, 1965, 1966; Kugler, 1965) and biochemically (Bayne, 1966, and in press), and it undergoes an elaborate maturation sequence (Lūsis, 1961; Smith, 1966). Because of such complexity one would suspect the existence of well developed controlling mechanisms, perhaps both endocrine and nervous. Our knowledge of such mechanisms is regrettably meagre (Laviolette, 1950a, 1954; Pelluet & Lane, 1961; Pelluet, 1964; Gomot & Guyard, 1964; Guyard & Gomot, 1964). Certainly external factors are also of great importance: the length of time spent in courtship and copulation necessitate suitable climatic conditions, while the sensitivity of the eggs to dessication (Bayne, personal communication) make important the selection of suitable egg laying sites and climatic conditions. Although many authors have stated the importance of such external factors, there is again little evidence of when and how they exert their effect.

Previous detailed work on pulmonate reproductive systems, especially their maturation, has been concerned with annual species. Perhaps the most thoroughly investigated species has been *Arion ater* (Abeloos, 1944; Lūsis, 1961, Smith, 1966) and the importance of internal and external factors has been stressed. *Arion* is normally an annual with a well defined breeding season, whereas *Agriolimax reticulatus* can apparently breed at any time of year, with up to 3 generations in a year. In spite of this difference in breeding cycle, the maturation stages of most of the reproductive tract are similar in both slugs. Thus the hermaphrodite gland, albumen gland, and common duct stages described by Smith (1966) for *Arion ater* are comparable with those described here for *Agriolimax reticulatus*. While Smith was able to subdivide the maturation of the penis and genital atrium of *Arion ater*, we were unable to do this for *Agriolimax reticulatus*. The major differences between the 2 reproductive tracts appear to be due to the greater separation in time of the male and female functions in *Arion ater*. Thus the hermaphrodite gland of *Arion ater* is nearly empty of sperm at the oöcyte stages, and the prostate gland starts to atrophy before the oviducal gland enlarges. This is in great contrast to maturation in *Agriolimax reticulatus*, where although the male system begins to mature first, both male and female systems appear to be functional in the mature animal. Also, copulation takes place some time before oviposition in *Arion ater*, whereas in *Agriolimax reticulatus* mature animals appear to be capable of both oviposition and copulation. Observations in this laboratory have, however, confirmed Luther's (1915) finding that *Agriolimax reticulatus* reared in isolation delay oviposition for a long time, and then lay very few eggs. Therefore, copulation appears to normally precede oviposition in *Agriolimax reticulatus*.

Although breeding did occur throughout the year (Fig. 2), there appeared to be optima in spring and autumn. Possibly the most obvious changes in climate at these times are fairly rapid and extensive temperature changes and high humidity. Carrick (1942) and Arias & Crowell (1963) have clearly shown that high environmental humidity is essential for oviposition in *Agriolimax* and, as we have observed large numbers of animals

in copula at dusk following a heavy shower, humidity may also be important in mating. Other possible evidence for the effect of external factors arises from the observation of variations in the relative quantities of sperm and ova in the spermatozoon, oöcyte and post-reproductive stages. This would imply that the timing and frequency of copulation and oviposition are not dependent on the stage of the hermaphrodite gland in these late stages. That some small animals were found with very mature hermaphrodite glands and some larger animals with immature hermaphrodite glands may imply that environmental factors affect the rate of development of this gland. Such variation could alternatively be due to genetic variation or physiological (Pelluet & Lane, 1961; Pelluet, 1964) or environmental (Bouillon, 1956; Richter, 1935; Rosenwald, 1927; Lūsis, 1966) influences on the relative quantities of the 2 types of gamete.

Laviolette (1950a, 1954), using very elegant experimental techniques, was able to demonstrate conclusively the existence of hormonal factors in the blood of various related arionid and limacid slugs, including *Agriolimax reticulatus*. These hormones apparently emanated from the hermaphrodite gland, and controlled the development of the albumen gland and the common duct but not of the penis. The site of production and nature of the hormone could not be determined. As shown in Figs. 8 and 10 and summarized in Table 1, the developmental stages of the common duct and albumen gland in *Agriolimax reticulatus* were closely related to the stage of the hermaphrodite gland. It is therefore likely that a similar endocrine control exists in this species.

In a very detailed study of growth in several species of Arionidae, Abeloos (1944) observed 3 phases of growth: an initial slow phase, the infantile stage, followed by a rapid phase, the juvenile stage, and lastly a slow one again, the mature stage. He also found that closely related changes took place in the reproductive system, an observation later amplified by Laviolette (1950b). The transition from the infantile to the juvenile stage (pre-puberty) was marked by oogenesis in the hermaphrodite gland and the rapid growth of the juvenile phase was paralleled by massive spermatogenesis. At the end of the juvenile phase the gonad had reached its maximum size. After the transition to the mature stage (puberty) there was marked growth of the reproductive tract. Smith (1966) has also noted that, at one stage in the development of the reproductive system of *Arion ater*, a large number of changes occurred in the tract; that stage would appear to coincide with Laviolette's transitional stage, i.e. at puberty.

In Table 1 our results for the changes observed in the histology of the reproductive tract of *Agriolimax reticulatus* throughout maturation, together with some observation on reproductive behaviour, are correlated to the stages of growth proposed by Abeloos (1944) and Laviolette (1950b) for the annual species of Arionidae. It can be seen that our results easily fit into such a scheme and, while not conclusive, suggest that the stages of growth in *Agriolimax* appear to be similar to those in the Arionidae.

In *Agriolimax reticulatus* there is thus some evidence that reproductive development may be controlled both by internal and by external factors. Experimental work is now needed to isolate the individual factors and to determine their effects.

ACKNOWLEDGMENTS

We are indebted to Professor F. W. Rogers Brambell, C.B.E., Sc.D., F.R.S., Zoology Department, University College of North Wales, for his help and encouragement. This work was supported by a grant from the Agricultural Research Council, U. K.

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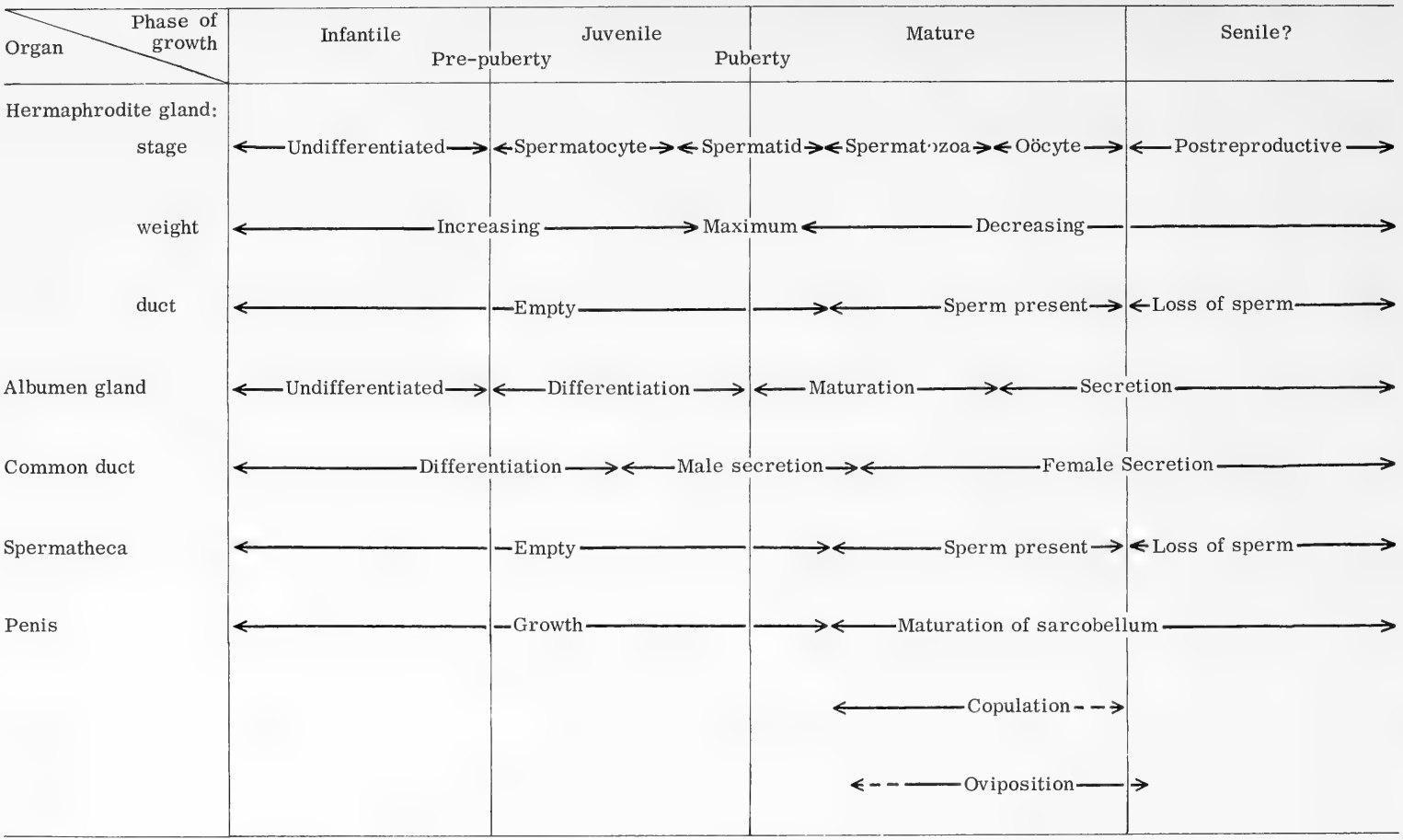
TABLE 1. *Agriolimax reticulatus*; Summary of reproductive development



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RÉSUMÉ

ETUDES SUR LA MATURATION DE L'APPAREIL REPRODUCTEUR D'*AGRIOLIMAX RETICULATUS* (PULMONATA: LIMACIDAE)

N. W. Runham et A. A. Laryea

Les *Agriolimax reticulatus* Müller ont été récoltés dans le Nord du Pays de Galles pendant une période de 18 mois. L'élevage a eu lieu tout au long de l'année avec une plus grande activité au printemps et à l'automne. L'appareil reproducteur des limaces a été disséqué et les différentes parties ont été séparément pesées et sectionnées. Le stade de maturation de la glande hermaphrodite n'est pas tout à fait en rapport avec le poids de l'animal. Le poids de cette glande atteint un maximum au "stade des spermatides" et ensuite décroît. Le sperme apparaît dans le canal hermaphrodite et dans la spermathèque à la fin de ce stade et y demeure ensuite jusqu'au stade qui suit la reproduction. La maturation de la glande à albumen et du canal commun est étroitement en rapport avec celui de la glande hermaphrodite. Les glandes du sarcobellum mûrissent au "stade des spermatozoïdes." Ces résultats semblent indiquer que les stades de maturation de l'appareil reproducteur sont en rapport avec les phases de croissance de l'animal et que des facteurs à la fois physiologiques et externes peuvent contrôler cette maturation.

RESUMEN

ESTUDIOS SOBRE EL ESTADO DE MADUREZ DEL SISTEMA REPRODUCTOR DE *AGRIOLIMAX RETICULATUS* (PULMONATA: LIMACIDAE)

N. W. Runham y A. A. Laryea

Agriolimax reticulatus fue colectado en Gales del Norte durante 18 meses. En todo el año se observaron crías que aumentaron al máximo en primavera y otoño. Se hizo la disección de los órganos reproductores y sus partes fueron separadas, pesadas y seccionadas. El peso del animal no demostró relación con el estado de madurez de la glándula hermafrodita; el peso de esta glándula aumenta a un máximo en el "estado espermático" y después se reduce. Al final de ese estado, apareció esperma en el ducto hermafrodítico y continuó presente hasta el estado post-reproductivo. La madurez de la glándula albuminoidea y ducto comun está estrechamente relacionada a aquella de la glándula hermafrodita. Las glándulas sobre el "sarcobellum" maduran en el "estado espermatozoico." Estas conclusiones parecen indicar que los estados de maduración del sistema reproductor se relacionan a las fases de crecimiento del animal y que los factores ambientales pueden controlar la maduración.

АБСТРАКТ

ИЗУЧЕНИЕ СОЗРЕВАНИЯ ПОЛОВОЙ СИСТЕМЫ У *AGRIOLIMAX RETICULATUS* (PULMONATA: LIMACIDAE)

Н. В. РАНХЕМ и А. А. ЛЕРАЙ

Втечение 18 месяцев в северном Уэльсе производились сборы *Agriolimax reticulatus*. Размножение происходило втечение всего года, с максимумами весной и осенью. Половая система улиток отпрепаровывалась, отдельные её части отделялись, взвешивались и делались срезы. Стадия созревания гермафродитной железы оказалась не очень связанной с весом животного. Вес этой железы достигал максимума на стадии "сперматиды", а затем начинал уменьшаться. Сперма появлялась в гермафродитном протоке и в семеприемнике в конце этой стадии и находилась там вплоть до конца периода размножения. Созревание альбуминовой железы и общего протока было тесно связано с созреванием гермафродитной железы. Железы на саркобеллуме созревают на стадии "сперматозоидов".

Всё это, видимо указывает на то, что стадии созревания репродуктивной системы этих моллюсков связаны с фазами их роста; на них могут влиять как физиологические факторы, так и условия среды.

MALACOLOGIA, 1968, 7(1): 109-116

AESTIVATION OF *BIOMPHALARIA GLABRATA* (BASOMMATOPHORA: PLANORBIDAE) GENETIC STUDIES

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ABSTRACT

Diapause and aestivation in *Biomphalaria glabrata*, associated with production of lamellae, constitute a mechanism for the survival of the species after drought and molluscicide. Self-fertilization of isolated lamellate individuals through several generations resulted in significant though unstable increases in average lamellae production up to 100% suggesting inheritance of the tendency. True breeding strains did not result. Matings between lamellate albinos and non-lamellate pigmented snails demonstrated transmission of the character by cross-fertilization. Results ruled out determination of the character by either simple recessive or simple dominant factors. It is concluded that the tendency for lamella formation and diapause in *B. glabrata* is determined by multifactorial inheritance.

The tendency of *Biomphalaria glabrata*[1] to climb spontaneously out of water and aestivate is a phenomenon significant for survival not only during the natural adversity of drought but also so as to survive man's application of aquatic molluscicides. This tendency for small, young *B. glabrata* for diapause was reported by Paraense (1957) in Brazil. Paraense also noted it was mostly snails with "apertural lamellae" that spontaneously climbed out of water. Association of lamellae with diapause and aestivation in *B. glabrata* was also described by Richards (1963, 1967). In these studies diapause is considered to be a period of spontaneous dormancy interrupting developmental activity and independent of environmental conditions, aestivation being prolonged survival out of water in a state of dormancy.

Several authors (Paraense, 1957; McCullough, 1958; Richards, 1963) have suggested that the formation of lamellae has a genetic basis. Information on the genetic aspects of this morphological character and on the associated phenomena of diapause and aestivation is pertinent to efficient control efforts in the field and analyses of results of experimental studies with *Biomphalaria glabrata* and other intermediate hosts of schistosomiasis.

METHODS

Taking advantage of the ability of *Biomphalaria glabrata* to reproduce by self-fertilization, and of the association of apertural lamellae with diapause behavior, 4 small lamellate snails were reared in isolation. The albino strain developed by Newton (1955) was used. Individual snails were reared in 400 ml beakers with Petri dish covers, in aerated tap water, and fed Romaine lettuce. The first 2 offspring of each of the snails to produce lamellae were

[1] *Australorbis glabratus* of the earlier literature.

isolated and reared and the procedure repeated.

Matings were carried out between lamellate albino progeny of snails selected for high-percentage lamellae formation and wild type, pigmented *Biomphalaria glabrata* of the same origin. Newton (1954) demonstrated that albinism in *B. glabrata* is transmitted as a single gene character recessive to wild type pigmentation. This character was employed as a marker in matings. The pigmented snails had failed to produce lamellate offspring by self-fertilization.

RESULTS

Selection for lamella production

Four clones were derived from 4 isolated lamellate parent snails, by successive selection and isolation of offspring that were allowed to reproduce by self-fertilization only. Two of the clones were discarded because of low egg production and high mortality. The other 2 were carried through 6 generations. The percentage of progeny with lamellae increased significantly in both series but did not become stabilized. Results of selection in one series (A) are shown diagramatically in Figure 1. The first snail to produce 100% (164/164) lamellate offspring, F_3 A-2, was one of a series showing a progressive increase in lamellate offspring in each generation: the parent snail, PA, produced 6% lamellate progeny; descendant F_1A produced 29%; F_2A-1 produced 72%. Of the 164 lamellate progeny of F_3A-2, 41 formed 2 or more sets of lamellae, a condition not uncommon when the frequency of lamella production is high. Six offspring of F_3A-2 were isolated, but produced only 0-73% lamellate offspring. Fifteen lamellate offspring of individual F_4 A-8 (with 73% lamellate progeny) produced 0-88% lamellate progeny. Snails F_3 A-3 and F_3A-4 each produced 99% lamellate offspring, isolations from which produced 0-100% lamellate progeny.

TABLE 1. Table summarizing F_1 results of 13 matings between lamellate albino and non-lamellate pigmented *Biomphalaria glabrata*.

Cross No.	F_1 progeny of non-lamellate pigmented parent % lamellate		F_1 progeny of lamellate albino parent % lamellate	
	Pre-cross self-fertilization	Post cross	Pre-cross self-fertilization	Post cross
1	0	7	50*	4
2	0	0	0	0
3	0	0	0	0
4	0	4	10	4
5	0	15	0	4
6	0	8	V-**	V-
7	0	0	16	0
8	0	0	73	0
9	0	3	23	67
10	0	0	12	0
11	0	0	88	0
12	0	0	13	0
13	V-	V-	0	0

* ___ albinos.

** V- no viable eggs laid.

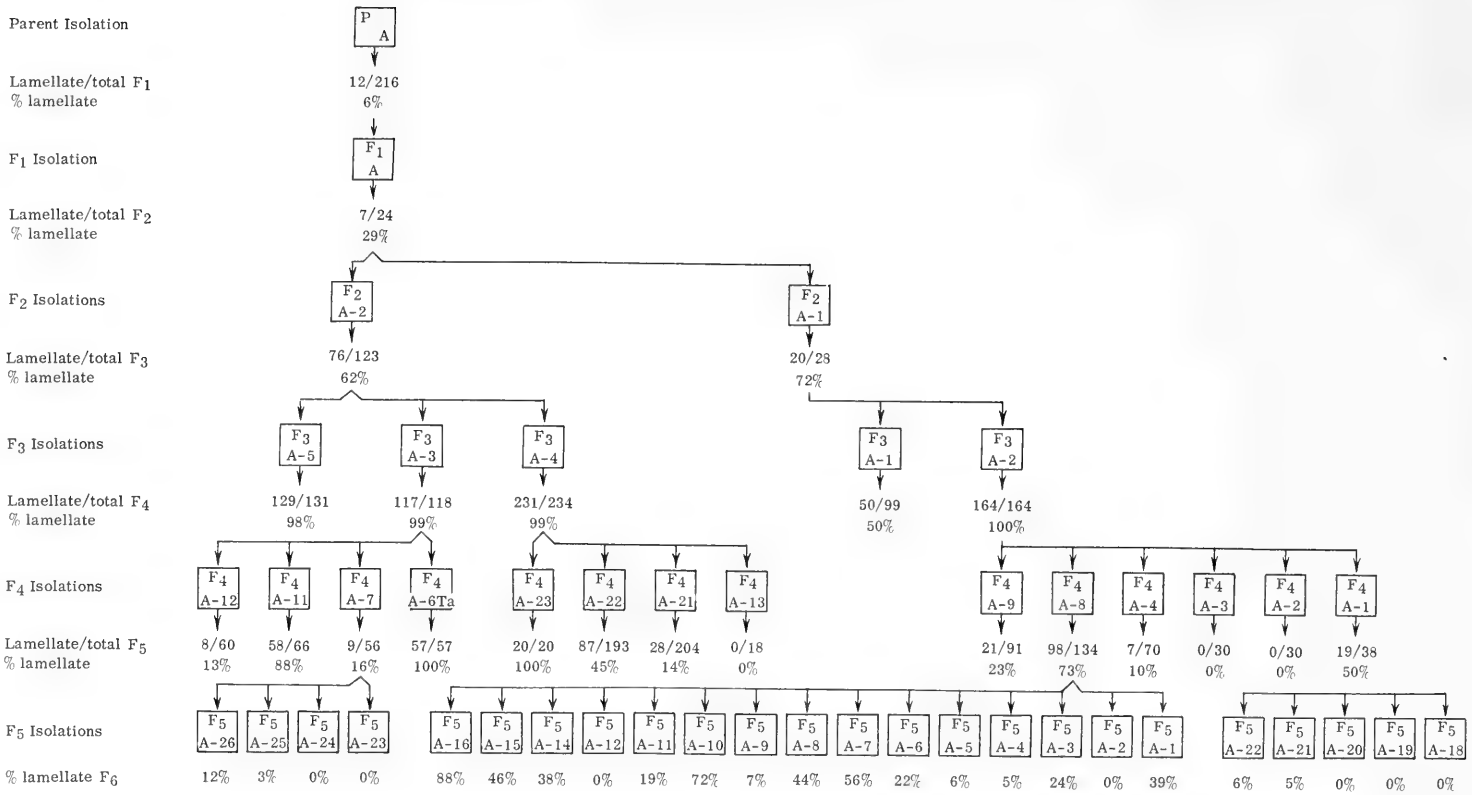

FIG. 1. Selection for lamella production by isolation and self-fertilization in *Biomphalaria glabrata*. Clone derived from one lamellate albino (A).

Mating experiments

Results of 13 matings between lamellate progeny of albinos producing over 90% lamellate offspring and non-lamellate wild type homozygous pigmented snails are summarized in Table 1. Ten of these albinos were F_4 generation snails from F_3A-2, F_3A-3, and F_3A-4, shown in figure 1. Five of the 13 pigmented parents produced lamellate F_1 progeny (3-15%) after mating. Each of these lamellate F_1 snails that was isolated and reared produced mixed pigmented and albino F_2 progeny (3:1) by self-fertilization, demonstrating that the F_1 snails were hybrids resulting from cross-fertilization. Albino parent No. 6 and pigmented parent No. 13 failed to produce viable offspring either before or after mating. Albinos No. 5 and 9 continued to self-fertilize after mating, but both fertilized their pigmented mates. Three of the albinos (Nos. 2, 3, and 13) failed to produce any lamellate offspring.

Results of cross No. 1 are shown in Figure 2. Before mating, self-fertilization of the lamellate albino parent produced F_1 progeny of which 50% had lamellae. Five of these were isolated and produced by self-fertilization 0-6% lamellate progeny. All the post-mating F_1 snails from both parents were pigmented, which demonstrated fertilization of the albino parent by the pigmented parent.

The pigmented parent produced 3/46 (7%) lamellate post-cross progeny, suggesting fertilization by the albino parent. Four (2 lamellate and 2 non-lamellate) F_1C-1 snails were isolated and reared. All 4 produced pigmented and albino F_2 progeny in 3:1 ratio, (demonstrating that the albino parent had fertilized the pigmented parent), all non-lamellate except one pigmented F_2 snail from lamellate F_1C-1-15. This individual, F_2C-1-15-14, was isolated and reared producing both pigmented and albino F_3 progeny, 9/34 (27%) being lamellate. Non-lamellate specimen F_1C-1-16, which produced no lamellate offspring by self-fertilization was mated with the original albino parent, after which it produced pigmented and albino progeny in 1:1 ration and 2/33 (6%) were lamellate.

The albino parent produced 6/163 (4%) post-cross lamellate F_1 progeny of which 7 (5 lamellate and 2 non-lamellate F_1 A-1 snails) were isolated and reared. All produced pigmented and albino F_2 progeny in 3:1 ratio. The 5 lamellate F_1 snails produced 0-16% lamellate F_2 progeny; the 2 non-lamellate F_1 snails 2% and 5%. Lamellate snail F_1A-1-6 produced 16% lamellate F_2 progeny of which 14 were isolated and reared. Six of these produced less than 10 viable F_3 offspring (not shown in Fig. 2). The other 8 included 1 albino and 7 pigmented F_2 snails of which 6 were heterozygous, producing mixed progeny, and 1 homozygous. Production of lamellate progeny by these 8 F_2 snails ranged from 0% to 91%. After self-fertilization, F_1A-1-6 was back-crossed with the original albino parent, after which it produced pigmented and albino offspring in 1:1 ratio of which 7/91 (8%) were lamellate. Lamellate individual F_1A-1-8, which produced no lamellate F_2 snails by self-fertilization, was back-crossed with the albino parent, after which it produced pigmented and albino offspring in the ratio 1:1, but none lamellate. Lamellate snail F_1A-1-9 produced 11% lamellate F_2 progeny. Three F_2 snails (2 lamellate and 1 non-lamellate) were isolated. The 2 lamellate F_2 snails were heterozygotes producing mixed offspring with 3% and 45% lamellate F_3 progeny. The non-lamellate F_2 was homozygous and produced 1/31 lamellate F_3 progeny. Lamellate F_1A-1-11 produced no lamellate F_2 progeny by self-fertilization, but after back-crossing with the original albino parent, it produced pigmented and albino offspring in 1:1 ratio, with 3/16 (19%) lamellate. Non-lamellate F_1A-1-12 produced 5% lamellate F_2 progeny by self-fertilization. Two of its non-lamellate pigmented F_2 snails were isolated. One was heterozygous and produced 0% lamellate F_3 progeny; the other a homo-

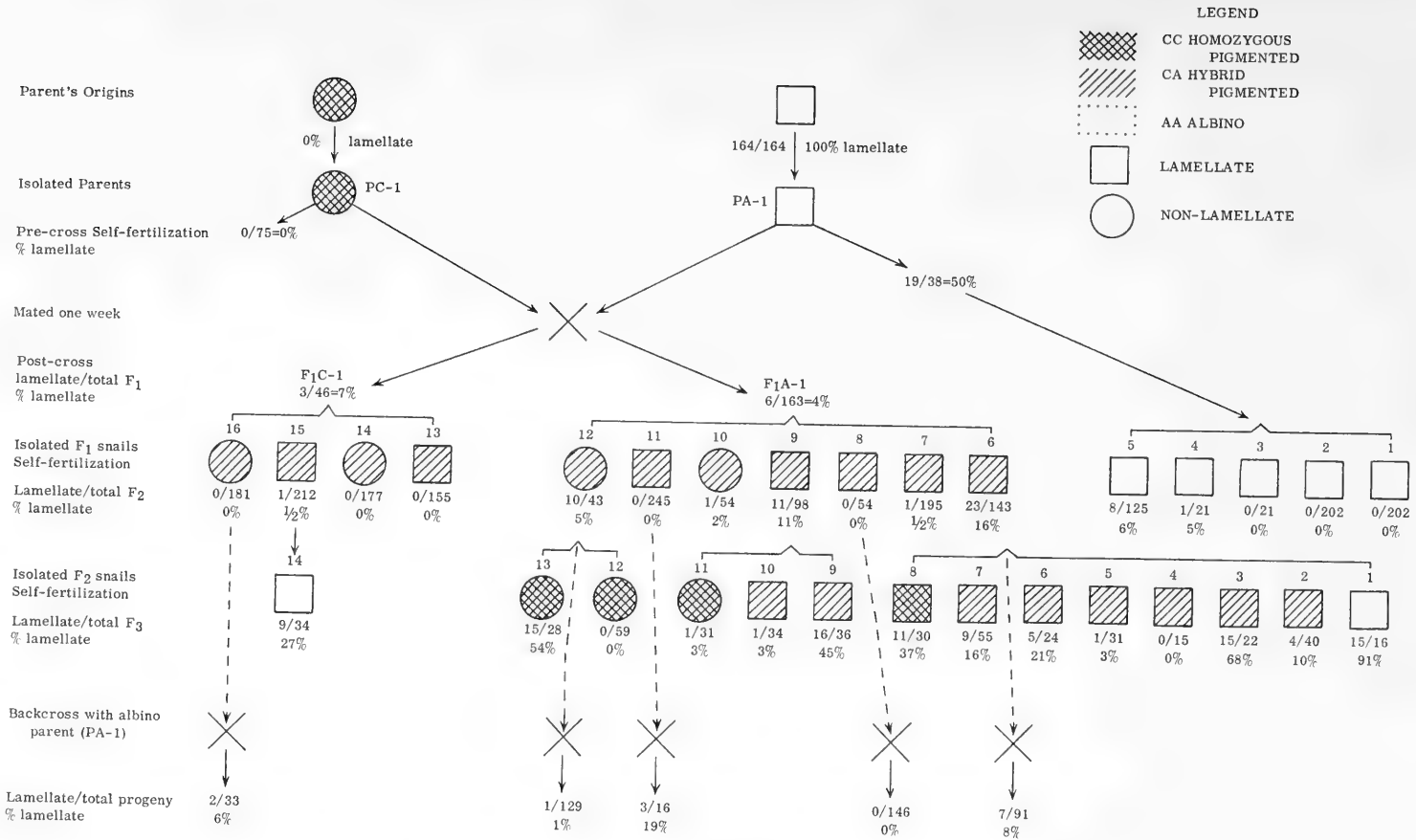

FIG. 2. Diagram of cross No. 1 between non-lamellate pigmented (C) and lamellate albino (A) *Biomphalaria glabrata*.

zygote produced 54% lamellate F_3 progeny. After mating with the albino parent F_1A-1-12 produced only 1/129 lamellate offspring.

DISCUSSION

Although true breeding strains were not obtained by selection (Fig. 1), the average occurrence of lamellate snails was greatly increased suggesting that inheritance is involved in the potential for lamella production. Gradual increase over several generations suggests multifactorial inheritance. As noted by Paraense (1957) and Richards (1963-1967), production of lamellae in *B. glabrata* is commonly associated with a tendency to climb out of water and aestivate; the lamellae also serve as supporting structures to the shell (Richards, 1964). Even in snails which do not climb out of the water, however, lamella production is associated with a delay in onset of egg laying (Richards, 1963). Thus, while lamella production and aestivation may insure survival of the population under adverse ecological conditions, a tendency to diapause in all individuals would represent a selective disadvantage in competition with other snails under favorable aquatic conditions. Where wet and dry seasons alternate, the optimum condition would appear to be a genetic mechanism to insure that part, but not all, of the population have a tendency and capacity for diapause and prolonged aestivation. This would insure survival through the dry season, yet rapid growth and early egg laying during the wet season. True breeding lamellate strains would therefore not be expected to be successful in nature, and extensive and prolonged laboratory experiments have failed to produce true breeding strains.

Occurrence of lamellate snails among the F_1 progeny from 5 of the pigmented parents after mating (Table 1) suggested that the potential for lamella production was transmitted from the albino parent in cross-fertilization. Appearance of lamellae after mating, in hybrid F_1 (Fig. 2) snails, suggested that the character is not determined by a simple recessive factor. Common appearance of lamellae in the offspring of non-lamellate snails by self-fertilization indicated that the trait is not determined by a simple dominant factor. The significance of the results obtained after employing the original albino parent as a male in 5 successive backcrosses is difficult to evaluate: production of lamellate progeny increased in 2 cases, decreased in 2, and remained 0% in the 5th.

It is concluded that the tendency to lamella formation and diapause in *Biomphalaria glabrata* is determined by multifactorial inheritance. It is probably a threshold character (Falconer, 1960) that can be influenced by a number of both genetic and nongenetic factors. The formation of lamellae in *B. glabrata*, and some other species of *Biomphalaria*, typically involves 1 set of 6 lamellae in a characteristic pattern (Richards, 1963). However, multiple sets of lamellae may occur, particularly with a high frequency of lamella production. Incomplete sets of lamellae in which only the parietal lamellae are formed are also not uncommon (Richards, 1963; Paraense, 1964). Since the occurrence of multiple sets of lamellae of one set, or of an incomplete set are all associated with thickening of the shell, deflection of the aperture to the left, and high frequency of diapause, such forms were all considered as "lamellate" in the present study. It is possible that these represent a series of thresholds, the quantitative analysis of which might help determine the number of gene pairs involved in the inheritance.

The capacity of *Biomphalaria glabrata* to escape the effects of molluscicides by a genetic tendency to climb out of water, independent of environmental conditions, and to aestivate, is pertinent to efficient control operations. The snails are of an inconspicuous size (1-6 mm diameter) when this tendency is expressed. Additional knowledge of the influence of genetics on behavior, growth,

reproduction, and various physiological characteristics is needed also to insure critical analyses of results in experimental studies with *B. glabrata* or other intermediate host snails.

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RESUMÉ

ESTIVATION DE *BIOMPHALARIA GLABRATA* (BASOMMATOPHORA: PLANORBIIDAE); ETUDES GENETIQUES

C. R. Richards

La diapause et l'estivation chez *Biomphalaria glabrata*,* accompagnée de la production de lamelles, constitue un mécanisme pour la survie de l'espèce à la sécheresse et à l'application de produits nocifs. L'autofécondation, pendant plusieurs générations, d'individus à lamelles isolés, a donné comme résultats des augmentations importantes mais irrégulières de la production moyenne de lamelles allant jusqu'à 100%, ce qui suggère l'hérédité du phénomène. Il n'en est pas résulté de véritables races physiologiques. Des croisements entre des albinos à lamelles et des individus pigmentés sans lamelles, démontrent la transmission du caractère par fécondation croisée. Les résultats permettent d'écarter la détermination du caractère par des facteurs simples, soit recessifs, soit dominants. On en conclut que la tendance à la formation de lamelles et à la diapause chez *B. glabrata* est déterminée par une hérédité multifactorielle.

* *Australorbis glabratus* de la litterature antérieure.

RESUMEN

ESTIVACION DE *BIOMPHALARIA GLABRATA* (BASOMMATOPHORA: PLANORBIIDAE); ESTUDIOS GENETICOS

C. R. Richards

La interrupción del crecimiento y estivación en *Biomphalaria glabrata, asociada con producción de lamelas, constituye un mecanismo de sobrevivencia a la sequía o los agentes moluscicidas. Autofertilización de individuos aislados, lamelados, por varias generacions, resultó en un significativo aunque inestable aumento en la producción media de lamelas hasta el 100%, lo que sugiere tendencia hereditaria. Cruzamiento entre individuos albinos lamelados, y pigmentados no lamelados, demostró la transición de caracteres. Los resultados descartan la determinación del carácter ya sea por los factores recesivos simples o dominantes simples. Se concluye que la tendencia a la formación de lamelas e interrupción del desarrollo en *B. glabrata* esta determinada por herencia multifactorial.**

* *Australorbis glabratus* de la literatura.

АБСТРАКТ

ЭСТИВАЦИЯ У *BIOMPHALARIA GLABRATA* (BASOMMATOPHORA: PLANORBIDAE) ГЕНЕТИЧЕСКОЕ ИССЛЕДОВАНИЕ

К. Р. РИЧАРДС

Диапауза и эстивация у *Biomphalaria glabrata* (Say) (=*Australorbis glabratus* прежних авторов) связана с образованием у них защитной слизистой пленки и представляют собою приспособление для выживания видов при осыхании и при действии моллюскоцидов. Самооплодотворение у изолированных особей, образующих пленку, на протяжении нескольких поколений дает в результате значительное, хотя и не стабильное, увеличение до 100% особей, образующих пленку, что позволяет предполагать наследование этой способности. Чистые линии не образуются. Скрещивание между образующими защитную пленку альбиносами и не образующими ее пигментированными особями, указывает на передачу этой способности, путем перекрестного оплодотворения. Результаты этого заставляют предполагать, что образование защитных пленок и диапауза у *B. glabrata* определяется множественными наследственными факторами, а не являются просто - рецессивными или просто - доминантными.

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ЗООГЕОГРАФИЧЕСКАЯ СТРУКТУРА И ИСТОРИЯ ФОРМИРОВАНИЯ ФАУНЫ НАЗЕМНЫХ МОЛЛЮСКОВ ТАЛЬША

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РЕЗЮМЕ

Наземная малакофауна Тальша (СССР, юго-восточное Закавказье), насчитывающая в своем составе 62 вида, может быть расчленена по зоогеографическому признаку на восемь группировок: голарктическую, палеарктическую, средиземноморскую, среднеевропейскую, малоазиатскую, кавказскую, армяно-иранскую и эндемичную гирканскую.

Детально обсуждается экологический облик представителей первых двух группировок; на основании этого анализа автор приходит к выводу о позднем вселении основной их массы в Тальш.

Для выяснения времени и возможных путей вселения моллюсков восстанавливается по литературным данным палеогеография района с момента появления его в виде острова (верхий палеоцен) до наших дней. Выясняются следующие временные возможности для вселения моллюсков: с Кавказа - со среднего миоцена по верхний плиоцен; со средней Европы и Средиземноморья - от верхнего миоцена по конец нижнего плиоцена; с Малой Азии - от нижнего миоцена по верхний плиоцен; с Восточной Азии - с нижнего миоцена по нижний плиоцен. Таким образом, налицо высокая степень изолированности района в течение всего времени его существования. В новейшее время территория лесного Тальша в отношении населяющей ее малакофауны полностью изолирована от прилежащих районов с востока - Каспийским морем, с севера - полупустынными пространствами Кура-Араксинской низменности, с юга и запада - аридными областями Иранского безлесного нагорья.

Анализируя состав эндемиков, автор приходит к выводу о их зоогеографической и генетической разнокачественности; гирканских эндемиков, по характеру их родственных связей, можно, в свою очередь, разделить на шесть групп: южноазиатско-тропическая, бореальная, среднеевропейская, средиземноморская, кавказская, малоазиатская.

Сравнивая качественный состав с некоторыми районами западной части Палеарктики, автор приходит к выводу о значительной обедненности фауны Тальша. Причины этого явления, очевидно, кроются в в неблагоприятных для моллюсков особенностях климата (характероной чертой которого является наличие засушливого летнего периода длительностью до 120 дней), в слабой кальцинированности почв, и, главным образом, в изолированности территории. Наличие высокого процента эндемиков (более 30%), обедненность фауны и некоторая

односторонность ее развития наводят на мысль об островном характере фауны Талыша. "...Современную фауну лесной части Талыша можно рассматривать как изолированное сообщество, сложившееся за сравнительно короткий отрезок времени под влиянием нескольких зоогеографических группировок и переработанное в небольшой степени в условиях своеобразного климата."

Далее отмечается назревшая необходимость унификации зоогеографических подразделений на основании сопоставления сведений, полученных разными авторами на разных группах организмов в одном районе.

В заключение, после критического анализа предложенных ранее вариантов зоогеографического районирования территории, автор предлагает вариант, наиболее отвечающий собранному материалу:

П А Л Е А Р К Т И Ч Е С К А Я О Б Л А С Т Ь
СРЕДИЗЕМНОМОРСКАЯ ПОДОБЛАСТЬ
Г и р к а н с к а я п р о в и н ц и я
Ленкоранский округ
А р м я н о - И р а н с к а я п р о в и н ц и я
Зувандский округ

"Под названием Талыш известна территория, лежащая в юговосточной части Азербайджана (СССР). Примерная площадь территории - 5370 кв.км. Естественные границы имеются на севере (Муганская степь, представляющая собой часть обширной Куро-Араксинской низменности) и на востоке (Каспийское море). На юге и западе границы более условны и проходят по нагорностепной части (позуванду), граничащей с аналогичными горными районами Ирана".

При первом ознакомлении со списком видов талышских наземных моллюсков бросается в глаза чрезвычайная пестрота их зоогеографического состава, связывающая более или менее отчетливо Талыш с разнообразными районами Палеарктики, а также высокая степень реликтовости фауны. К аналогичному выводу пришли исследователи, занимавшиеся талышскими представителями других групп живых организмов: амфибиями и рептилиями (Н.И.Соболевский, 1929), птицами и млекопитающими (М.А.Мензбир, 1934), муравьями (К.В.Арнольди, 1948), некоторыми жуками (И.Г.Самедов, 1963), а также расмеи (А.А.Гроссгейм, 1926, В.Г.Левандовский, 1899, В.П. Малеев, 1938, Л.И.Прилипко, 1954 и др.). Приэтом на разных группах организмов вопрос о генезисе фауны Талыша решался по-разному, т.к. в каждой группе обнаруживался свой процент реликтовости и удельный вес вполне закономерно, ибо каждой группе животных и растениий свойственна разная иммиграционная способность, разная стапень приспособляемости и разный уровень общей организации. Именно в силу малой подвижности наземных моллюсков, положительной стенобионтности большей их части, неспособности преодолевать многие географические барьеры эта группа дает надежный и удобный материал для решения зоогеографических проблем.

Рассмотрим зоогеографическую структуру современной малакофауны Талыша. Здесь мы наблюдаем представителей восьми зоогеографических группировок.

1. Голарктические виды: *Cionella lubrica* (Müll.), *Vallonia puchella* (Müll.), *V. costata* (Müll.), *Punctatum pygmaeum* (Drap.), *Euconulus fulvus* (Müll.), *Zonitoides nitidus* (Müll.), *Columella edentula* (Drap.). (7 видов).

2. Палеарктические виды: *Carychium minimum* Müll.

3. Средиземноморские виды: *Pomatias rivulare* (Eichw.), *Lauria cylindracea* (Da-Costa), *Vertigo pygmaea* (Drap.), *Truncatellina strobeli* (Gredl.), *Acanthinula aculeata* (Müll.), *Punctum micropleurum* (Paget), *Milax caucasicus* (Simr.), *Helicella krynickii* (Kryn.), *H. derbentina* (Kryn.), *Helix lucorum* L., *Oxychilus subeffusus* (Bttg.). (11 видов).

4. Среднеевропейские виды: *Vertigo pusilla* Müll., *Orcula doliolum* (Brug.), *Truncatellina costulata* (Nilss.), *Ena obscura* (Müll.), *Vitrina annularis* (Stud.). (5 видов).

5. Малоазиатские виды: *Chondrula tridens* (Müll.), *Jaminia pupoides* (Kryn.), *J. isseliana* (Iss.), *Zebrina hohenackeri* (Pfr.), *Gigantomilax koenigi* (Simr.). (5 видов).

6. Кавказские виды: *Vitrea contortula* (Kryn.), *Oxychilus disciformis* Riedel, *Ox. sieversi* (Bttg.), *Ox. elegans* (Bttg.), *Deroceras caucasicus* (Simr.), *D. caspius* Simr., *D. melanocephalus* (Kal.), *Parmacella olivieri* Guv., *Euomphalia pisiformis* (Pfr.), *E. ravergieri* (Fer.). (10 видов).

7. Армяно-Иранские виды: *Mucronaria gustavi* (Bttg.), *Pupilla signata* (Mouss.). (2 вида).

8. Эндемичные гирканские виды: *Caspicyclotus sieversi* (Pfr.), *Carychium lederi* (Bttg.), *C. primitivium* sp. n., *Succinoides stelliferus* gen. n., sp. n., *Pagodulina lederi* (Bttg.), *Serrulina sieversi* (Pfr.), *Caspiophaedusa perlucens* (Bttg.), *Euxina talyschana* Likh., *Oxychilus caspius* (Bttg.), *Ox. filicum* (Kryn.), *Trochovitrina lederi* (Bttg.), *Limax keyserlingi* Martens, *Gigantolimax talyschanus* Simr., *G. lenkoranus* Simr., *Lytopelte maculata* (Koch & Heyn.), *Trigonochlamys bicolor* (Simr.), *Tr. sphingiformis* (Simr.), *Theba longiflagellata* sp. n., *Th. talyschana* (Mart.), *Th. maxima* sp. n., *Caucasotachea lencoranea* (Mouss.). (21 вид).

Остановимся вначале на распространении в Талыше представителей двух первых зоогеографических групп (их обычно называют широкораспространенными).

Все эти виды занимают экологические ниши, в общем идентичные тем, которые ими заселяются в большей части мест их ареала, в том числе и в умеренных широтах. Мы не найдем ни одного из них ни в "ленкоранских джунглях", ни в зарослях третичного реликта - железного дерева (*Parrotia persica*), ни в иных экзотических ассоциациях; лишь изредка некоторые из них попадаются в подстилке лесов энлемичного каштанолистного дуба и граба (напрумер, *Punctum pygmaeum* (Drap.), экологический спектр которого вообще очень широк). Основные биотопы, которые они заселяют - это поймы рек, преимущественно в нижнем течении, у уреза воды, в кучах речных выбросов, на береговом валу, в пойменных и припойменных ольшанниках, по опушкам сырых лесов на низменности, составленных той же ольхой (часто с примесью карагача и некоторых других пород). Те из видов, которые поднимаются в аридную нагорно-степную зону (например, *Vallonia pulchella* (Müll.), обитают в прикорневых частях трав и кустарников или под камиями, близ открытой воды или в местах выхода грунтовых вод, чем достигается резкое уменьшение влияния на них аридного климата нагорья и нивелируется разница в микроклимате низменности, горных склонов и нагорья.

Следовательно, эти виды отнюдь не перестроились в сторону большей сухоустойчивости, которая, казалось бы, требовалась от них в условиях талышского климата (характерной чертой которого является наличие засушливого сезона продолжительностью до 120 дней), но просто среди широкого ассортимента условий этого необычного для них района выбрали для своего обитания те, которые в максимальной степени соответствуют таковым в умеренных широтах.

Для дополнения экологической характеристики талышских представителей широкораспространенных видов необходимо отметить их малую количественную встречаемость. При условном[1] разделении всей малакофауны на пять категорий по признаку частоты встречаемости широкораспространенны видов.

Это говорит о том что в составе фауны данная группа играет далеко не ведущую роль; присовокупляя предыдущие материалы по этоий зоогеографической группировке, мы приходим к выводу о сравнительно позднем вселении основной массы ее элементов в Талыш.

Когда и какими путями могло осуществиться это вселение? Таких путей два: прямой - через Кавказский перешеек, и окружный - через западную Европу, возможно, северную Африку (Гибралтарский пролив образовался сравнительно очень недавно) и Малую Азию.

Прямая связь Кавказа с южнорусской сушей открылась лишь в нижнем плиоцене в результате регрессии конца понтического времени (Н.И. Андрусов, 1918). М.В.Муратов (1951) предполагает, что имело место еще одно кратковременное соединение понто-каспийского бассейна (куяльницкий и акчагыльский бассейны).

С другой стороны, к Малой Азии весь Кавказ присоединяется уже в среднем миоцене (Б.П.Жижченко, В.А.Колесников, А.Г.Эберзин, 1940), а Эгейская суша - мостик между Малой Азией и более западными районами Средиземноморья - существовала вплоть до плейстоцена, после чего испытала частичное погружение.

Экологический облик рецентных представителей широкораспространенных видов, их небольшая численность - как в качественном, так и в количественном отношении - говорят как будто бы за большую приемлемость первого варианта; но, как будет видно дальше, на самом деле имело место осуществление обоих вариантов.

Ценные сведения о генезисе древесных палеарктов мы находим у А.Н. Краснова (1911). Указанный автор подчеркивает, что в палеоцене по всей Европе была распространена флора полутропической страны с климатом равномерно теплым и влажным; те из листопадных растений, которые были здесь представлены, являются предшественниками флоры умеренной полосы Старого Света: буки, березы, дубы, тополи, ясени, первичные липы. Причем первичные дубы обнаруживают поразительное сходство с ***Quercus castanaefolia C.A.M.*** и ***Q. pontica Koch*** - эндемиками соответственно Талыша и Колхидской низменности. Другими словами, корни большинства современных европейских древесных пород были заложены уже в палеоцене. Будет вполне обоснованным, на наш взгляд, предположить то же самое применительно к наземным моллюскам.

Для рассмотрения более конкретно во времени и в пространстве процесса вселения моллюсков на территорию гирканской зоогеографической провинции выясним, насколько велика была степень изоляции этой территории начиная с того момента, когда Талыш впервые поднялся из вод

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[1] 1 категория - встречены более чем в 40% всех проб.
2 категория - встречены в 33-19% всех проб
3 категория - встречены в 12-5% всех проб
4 категория - встречены в 4-2% всех проб
5 категория - единично найденные виды.

вернепалеоценового моря.[2] Тогда это был небольшой скалистый островок вулканического происхождения. Примерно в то же время или несколько раньше оформляется Малый Кавказ как самостоятельная орографическая единица. Так как к тому времени на Земле существовали уже почти все ныне живущие семейства наземных моллюсков, и ни одно из них не имеет Тальш своим центром распространения,[3] то скорее всего, в этот период малакофауна в Талыше отсутствовала. В конце нижнего миоцена, вследствие поднятий, обеспечивших соединение Кавказа с Малой Азией, могло произойти первое вселение восточно- и малоазиатских элементов как на Кавказский полуостров в целом, так и в юго-восточную его часть. Но вскоре юго-восточное направление перерезается трансгрессией, соединившей Каспийскую впадину с Индийским океаном на месте нынешнего Персидского залива, и, как след былой связи, остался один из самых древних вселенцев - *Caspicyclotus sieversi* (Pfr.).

Картина развития этой группы рисуется, по нашим представлениям, следующим образом. Выход из моря предковых форм осуществился в юго-восточной Азии на границе мезозоя и кайнозоя. Затем, в палеоцене, имело место стремительное завоевание Евразии. Доказательством тому служат многочисленные находки в Европе эоценового *Palaeocyclotus* Fisher, к которому весьма близка и талышская рецентная форма. Затем этот эоценовый вид на большей части своего ареала вымер; *Caspicyclotus* Forcart мы рассматриваем как его прямого потомка. Развитие же этой группы в юго-восточной Азии продолжалось в ином направлении и более быстрыми темпами, в результате чего азиатские формы имеют ряд прогрессивных черт по сравнению с *Caspicyclotus sieversi*, и образуют пышный букет родов и видов. Разницу в скорости эволюции мы склонны усматривать в очень малой климатической измененности Тальша по сравнению с территорией основного ареала группы. Подтверждением древности вида является его тесная связь с третичным реликтом растительного царства - железным деревом: в чистых зарослях этой породы плотность данного вида достигает иногда 120 экз./кв.м., в то время как в остальных типах лесов эта величина не поднимается выше 40-50, либо этот вид вообще отсутствует.

Вернемся к поставленному вопросу. Миграционный процесс с другой стороны - с запада в основном проходил за сравнительно небольшой промежуток времени - от середины миоцена до начала плиоцена.

Несмотря на продолжающуюся формальную морфологическую связь с Малой Азией, с начала - середины плоцена процесс вселения западных и юго-западных элементов вначале резко сократился, а затем вообще почти прекратился: нарастание аридности климата к югу и западу от Тальша закрыло дорогу с этого направления всем мезо- а тем более гигрофилам; что же касается климата самого Тальша, то он остался почти неизмен-

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[2] Приводимые здесь сведения по геологической истории района опираются на данные Страхова, 1948, Андрусова, 1818, Жижченко, Колесникова, Эберзина, 1940, Маркова, Лазукова, Николаева, 1965, Мензбира, 1934, Муратова, 1951 и др.

[3] Не совсем ясным остается вопрос о **Trigonochlamydidae**; некоторые соображения на этот счет приводятся ниже, при обсуждении эндемичных форм.

ным благадаря близости теплого моря.

С начала плиоцена и до наших дней Талыш фактически находится в состоянии изоляции: с востока он омывается морем; с юга и запада к верхней границе лесов могут проникнуть по полупустынному засушливому нагорью лишь генетически молодые сухоустойчивые виды, но не получены еще доказательства (и вряд ли когда-нибудь будут получены) обратного процесса - превращения сухоустойчивых видов в мезофильные; эти виды действительно дошли до Талыша, но были остановлены верхней границей лесов. С севера изоляция осуществлялась неоднократными взаимозамещениями каспийских трансгрессий (акчагыльская, апшеронская, бакинская, нижнехазарская) и полупустынных аридных областей. Современные Муганская и Сальянская степи - окончательный продукт этих процессов - делают невозможным новейшее вселение северных мигрантов. Единственный вид, связывающий Ленкоранскую низменность с более северными районами - ***Helicella krynickii*** (Kryn.) - очень сухоустойчив, и поэтому лишь он мог проникнуть через эти засушливые пространства.

Следовательно, в верхнем миоцене - периоде интенсивного заселения Талыша - три потока переселенцев (часть широкораспространенных, средиземноморские, европейские) сливались в одно русло на территории Эгейской суши и Малой Азии, принимая здесь и местные элементы. В нижнем плиоцене этот поток был прерван. В постплиоцене заселялась лишь часть Талыша выше лесной зоны за счет молодых азиатских ксерофилов, которые в Зуванде в силу своего колоссального количественного развития создают иллюзию богатства фауны: на самом же деле фауна Зуванда качественно очень бедна и составлена в основном шестью видами: ***Zebrina hohenackeri*** (Pfr.), ***Helicella derbentina*** (Kryn.), ***Jaminia pupoides*** (Kryn.), ***J. isseliana*** (Iss.), ***Mucronaria gustavi*** (Bttg.) и ***Pupilla signata*** (Mouss.). Приэтом основной фон создают только три первых вида, а последний вообще встречается очень редко. Кроме того, здесь, как уже отмечалось, имеются некоторых виды из числа широкораспространенных, но они населяют лишь очень влажные микробиотопы близ рек и заболоченных мест.

Что касается ***Mucronaria***, то среди клаузилиид Талыша она является наиболее молодым элементом, также связанным своим происхождением с Малой Азией (главным образом, с центральными и северными ее частями), и проникла в Талыш, вероятно, уже в постплиоцене, вместе с остальными ксерофилами. Этот вид живет в расселинах скал и в осыпях, и в лесную зону никогда не спускается.

Рассмотрим взаимоотношения фаун Талыша и Кавказа. До наступления засушливого периода гирканская фауна, несомненно, была широко связана с Кавказом; более того, вполне возможно, что территория Талыша не представляла собой конечную точку вселения, но являлась скорее перевалочным пунктом для видов, направляющихся на Кавказ; но там к тому времени уже имелась своя, гораздо более богатая фауна, вступившая в экологическую конкуренцию (Лэк, 1957) с пришельцами; после разрушения упомянутой связи между Кавказом и Талышом большая часть гирканских видов на Кавказе вымерла и, как след прошлых связей, остались разорванные ареалы ***Serrulina sieversi*** (Pfr.), ***Caspiophaedusa perlucens*** (Bttg.), ***Trochovitrina lederi*** (Bttg.); ***Caspicyclotus*** также встречается в некоторых районах Грузии (Сигнахи, Лагодехи).

Особый интерес представляют собой гирканские эндемичные формы. Рассматривая родственные связи отдельных видов, мы пришли к убеждению, что соединение их в одну группу "эндемиков" является чисто формальным

и в данном случае лишь указывает на тот факт, что эти виды нигде (или почти нигде) более не встречаются, являясь непременной принадлежностью данного района. Но в то же время эта группа весьма разнокачественна по своей сущности: среди них мы различаем эндемиков более старых и более молодых; эндемиков, имеющих связь и с западом, и с востоком, и с севером. Аналогичную мысль высказывает И.Г.Пидопличко (1957): "Реликты нужно различать не только по возрасту, но и по степени их идентичности с предшествующими формами".

Гирканских эндемиков, по характеру их родственных связей, которые в большинстве случаев более или менее ясно проявляются, можно, в свою очередь, разделить на следующие зоогеографические группы:

1. Южноазиатско-тропическая: ***Caspicyclotus sieversi*** (Pfr.), ***Serrulina sieversi*** (Pfr.), ***Caspiophaedusa perlucens*** (Bttg.), ***Lytopelte maculata*** (Koch & Heyn). (4 вида).
2. Бореальная: ***Carychium lederi*** (Bttg.), ***C. primitium*** sp.n. ***Succinoides stelliferus*** gen.n., sp.n. (3 вида).
3. Среднеевропейская: ***Pagodulina lederi*** (Bttg.).
4. Средиземноморская: ***Trochovitrina lederi*** (Bttg.), ***Gigantomilax talyschanus*** Simr., ***G. lencoranus*** Simr., ***Theba longiflagellata*** sp.n., ***Th. talyschana*** (Martens), ***Th. maxima*** sp.n., ***Caucasotachea lencoranea*** (Mouss.). (7 видов).
5. Кавказская: ***Limax keyserlingi*** Mart., Trigonochlamydidae. (3 вида).
6. Малоазиатская: ***Euxina talyschana*** Likh., ***Oxychilus filicum*** (Kryn.), ***Ox. caspius*** (Bttg.). (3 вида).

Таким образом, среди наземных моллюсков Тальша как будто нет чисто автохтонных форм, хотя мы и наблюдаем здесь таких древних реликтов, как ***Caspicyclotus sieversi*** (Pfr.), ***Succinoides stelliferus*** gen.n., sp.n. - представителей монотипических родов.

Если истрия появления в Тальше первого из этих видов в общем ясна и изложена выше, то со вторым вопрос не столь прост. Наличие чехла пениса у этого вида говорит о сравнительной его молодости (П.В.Матекин, 1960); то обстоятельство, что на данной территории видообразрвательный процесс коснулся этой формы настолько, что привел к образованию нового рода, говорит о том, что она появилась на данной территории давно. Но все же, повидимому, группа янтарок, имеющих чехол пениса (***Succinea*** s. str., по П.В.Матекину, 1956,1960), в миоцене уже была сформирована и имела весьма широкое распространение, о чем говорит ее современный ареал. Поэтому нам думается, что предковые формы ***Succinoides*** проникли в Тальш с юго-запада вместе с первыми же вселенцами. Кроме того, следует отметить, что огромный род ***Limax*** представлен в Тальше лишь одним видом, который справедливо возводится в ранг подрода (***Caspilimax***).

История гирканских видов Clausiliidae разработана И.М. Лихаревым (1957, 1962). В первую очередь обращает на себя внимание тот факт, что всего четыре найденных нами в Тальше вида клаузилиид принадлежат к трем подсемействам из пяти, представленных в Советском Союзе, что уже свидетельствует о их зоогеографической и генетической разнокачественности.

Повидимому, вначале, еще в среднем миоцене, из юго-восточной Азии в Тальш попали оба представителя Phaedusinae и некогда были распротранены по всему Кавказу, о чём свидетельствует тот факт, что они до сих пор встречаются отдельными пятнами к югу от Главного Кавказского хребта. Близкие к ним виды в настоящий момент населяют тропические леса юго-восточной Азии. Кроме того, А.А.Стекловым (1963) была найдена ***Serrulina ex gr. sieversi*** в верхнем миоцене (сармат) Предкавказья.

Euxina имеет своих ближайших родственников в Малой Азии; очевидно, этот вид проник из указанного района в Талыш вместе с **Phaedusinae** или несколько позже, но не с юго-востока, а с юга и юго-запада, в составе основной массы среднеевропейских и средиземноморских мигрантов.

Одним из наиболее западных вселенцев является *Trochovitrina*, которая имеет своих ближайших родственников на Канарских островах; появление её на Кавказе следует, вероятно, датировать нижним плиоценом.

Присутствие в Закавказье, Талыше и северных областях Ирана представителей эндемичного для этих мест семейства **Trigonochlamydidae** связывает между собой эти территории; но, с другой стороны, подсемейство **Trigonochlamydinae** представлено в Закавказье и в Талыше, а подсемейство **Parmacellillinae** -в северной части Ирана. Это говорит о том, что эти области изолированы друг от друга уже давно; и если предполагать средиземноморское происхождение этой группы, то изоляция наступила до того, когда нарушилась связь Талыша с более западными районами.

Более вероятно однако, что семейству следует приписать гирканское происхождение; то, что на Кавказе, обитает значительное число его представителей, может быть объяснено слабой экологической конкуренцией, ибо эти слизни являются хищниками и нередко попадаются в средних и верхних слоях почвы, а не на её поверхности. Другие же хищные виды моллюсков на Кавказе очень немногочисленны. Окончательное решение вопроса о генезисе группы может быть осуществлено лишь после таксономической ревизии всего семейства. Именно поэтому данная группа включена в кавказскую зоогеографическую группировку пока условно.

Изложенные выше материалы подводят нас к мысли об островном характере наземной малакофауны Талыша. Об этом свидетельствуют такие признаки, как её обедненность; нерезко выраженная, но все же вырисовывающаяся, односторонность; высокий процент эндемиков.

Приведем сведения по качественному составу малакофауны некоторых территорий западной части Палеарктики:

Англия	106 видов
Германия	около 200
Сицилия (Шарфф)	250
Крит (Шарфф)	121
Крым (Лихарев)	81
Врсточная Грузия (Лежава)	120
Подмосковье (Малевич и Старобогатов)	75
Талыш (по нашим данным)	62

Внесем в эти данные некоторые коррективы. Сведения Шарффа относятся к тому периоду в развитии малакологии, когда большинство систематиков пользовалось еще чисто конхиологическим методом, в результате чего виды нередко описывались на основании находки нескольких пустых раковин, обнаруживавших какие-то отклонения от уже известных форм, причём природа этих отклонений, как правило, оставалась невыясненной. Если же применить концепцию о полиморфных видах, то очень возможно, что эти данные придется несколько сократить. Тоже самое можно в известной мере отнести к цифрам, представленным Гейером. В работе И.И. Малевича и Я.И. Старобогатова указываются все без исключения виды, которые хоть раз были найдены в Московской области; в их числе находятся и сомнительные, и явно интродуцированные; таких видов наберется 13-14.

Но даже после этих уточнений обедненность талышской малакофауны

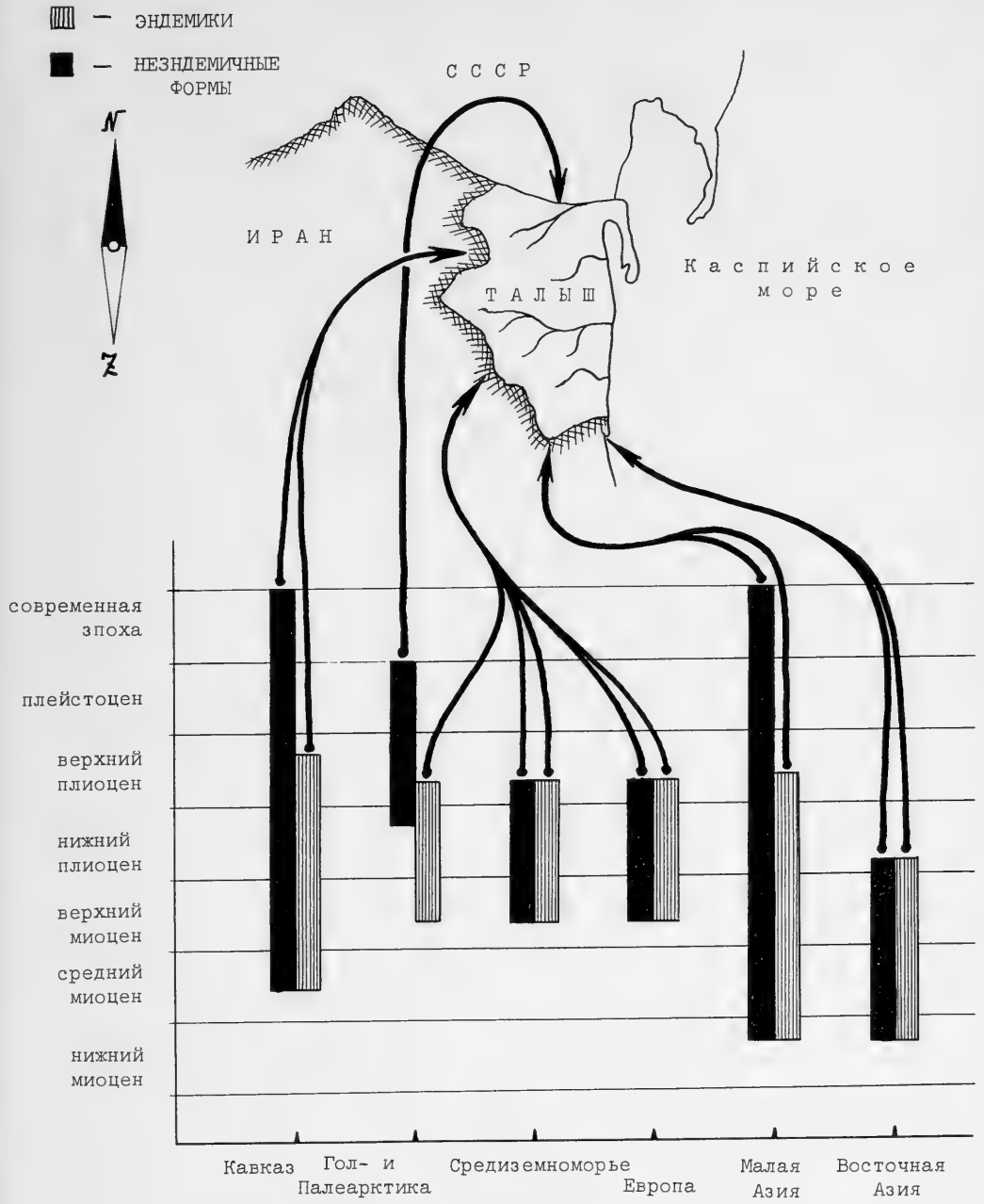

ТАБЛИЦА 1. Схема зоогеографических связей малакофаунн Тальша во времени и в пространстве (Видно, что в настоящее время малакофауна Тальша связана с фаунами Кавказа и Малой Азии. Характер этих связей рассматривается в тексте).

FIG. 1. A scheme of zoogeographical bonds of Talysh mollusk fauna in time and space. It is apparent that at present the mollusk fauna of Talysh is connected to the faunas of Caucasus and Asia Minor. The character of these bonds is discussed in the text.

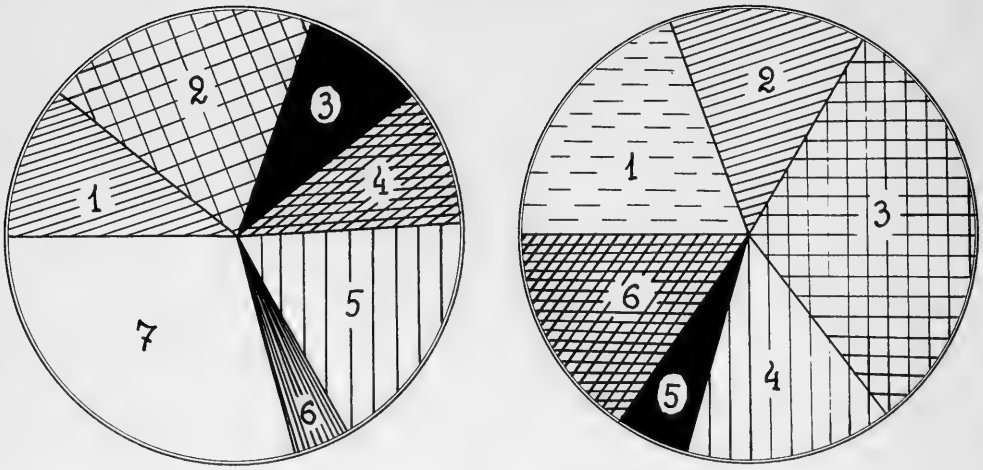

ТАБЛИЦА 2. Слева: Зоогеографический состав наземной малакофауны Тальша. 1 - широкораспространенные виды. 2 - средиземноморские виды. 3 - среднеевропейские виды. 4 - малоазиатские виды. 5 - кавказские виды. 6 - гирканские эндемичные виды.

Справа: Зоогеографические связи гирканских эндемиков. 1 - виды юговосточноазиатского происхождения. 2 - виды бореального происхождения. 3 - виды средиземноморского происхождения. 4 - виды кавказского происхождения. 5 - виды европейского происхождения. 6 - виды малоазиатского происхождения.

FIG. 2. Left: Zoogeographical composition of terrestrial mollusk fauna of Talysh; 1, widely distributed species; 2, Mediterranean species; 3, Middle European species; 4, Asia Minor species; 5, Caucasian species; 6, Hyrcanian endemic species.

Right: Zoogeographical bonds of Hyrcanian endemic species. 1, Species of south-eastern Asiatic origin; 2, Species of boreal origin; 3, Species of Mediterranean origin; 4, Species of Caucasian origin; 5, Species of European origin; 6, Species of Asia Minor origin.

вырисовывается достаточно ясно, особенно, если учесть его географическое положение: эта территория расположена почти у 20 параллели, чуть севернее ее. Восточная Грузия примерно на той же широте. Сицилия и Крит ограничены 20 параллелью с севера. Остальные же территории, кроме Крыма, расположены на 2-3 тысячи километров севернее.

И.И.Пузанов (1927), посвятив свою работу изучению моллюсков Крыма, формулирует закон "островного обеднения": "...Фауна всякого острова, являющегося остатком более крупной материковой массы, с течением времени неминуемо сокращает свой состав. Повидимому, в этом фатальном оскудении островных фаун играют роль и сокращение пригодных для обитания стаций, и размножение в тесных родственных пределах, и в особенности - невозможность для отдельных элементов фауны регенерировать после каких-либо пертурбаций климатического и иного характера, проходящих безнаказанно на материке". Напомним, что обедненная фауна

Крыма составлена 81 видом; в то же время фауна более южной лесной области - Талыша - насчитывает почти на 20 видов менее!

Попытаемся теперь выяснить, в чем заключается однобокое развитие талышской фауны. Здесь, правда, нет групп, получивших явное преимущественное развитие, как это имеет место в Крыму. Но такие крупные группы, как **Pupillidae** и **Clausiliidae** представлены единичными видами, в то время как **Zonitidae, Limacidae** и **Helicidae** играют значительно более заметную роль.

Что же касается главной особенности фауны - высокого процента эндемиков, то эта сторона не нуждается в комментариях.

Ниже уже отмечалась изолированность Талыша. В самом деле, оживленный обмен фаунами мог происходить лишь в небольшой промежуток времени - со среднего миоцена по середину верхнего плиоцена-с Кавказом, с верхнего миоцена по нижний плиоцен-со Средиземноморьем и Европой, с конца нижнего миоцена по верхний миоцен - плиоцен-с Малой и Восточной Азией. Бореальные формы могли проникнуть с нижнего плиоцена по плейстоцен (большая часть видов) прямым путем с севера, и с конца миоцена по начало плиоцена - кружным путем с юго-запада. Этим путем шел, вероятно, ***Punctum pygmaeum*** **(Drap.)**, о чем говорит экологический облик талышских его представителей.

На таблице 1 графически показан процесс заселения Талыша во времени и в пространстве: зачерненные столбики касаются всех неэндемичных видов. Процесс вселения предков эндемичных форм обозначен отдельно заштрихованными колонками. Таблица 2 иллюстрирует удельный вес каждой из зоогеографических группировок в общем составе фауны (левая диаграмма) и удельный вес зоогеографических группировок среди эндемичных форм.

В настоящее время связь лесного Талыша (как советской, так и иранской его части) с остальными лесными областями отсутствует. Нагорно-степная зона Талыша, как уже упоминалось, связана с молодой ксерофильной фауной Иранского нагорья, но эта связь никак не отражается на фауне лесных районов. Самостоятельность степного комплекса и его сравнительную молодость подчеркивает и В.В.Кучерук (1957), опираясь на данные по млекопитающим.

Таким образом, современную фауну лесной части Талыша можно рассматривать как изолированное сообщество, сложившееся за сравнительно короткий отрезок времени под влиянием нескольких зоогеографических группировок и переработанное в небольшой степени в условиях своеобразного климата, одной из важнейших черт которого в данном случае является периодическая смена дождливого и засушливого периодов.

Приведенные в настоящей работе данные являются подтверждением воззрения, предложенного Гуликом (**Gulick**, 1905), исследовавшим изменчивость раковин наземных моллюсков: изоляция есть один из ведущих факторов видообразования. Обеспечивается изоляция морскими водами, полосой безводной полупустыни или скалистыми хребтами - это значения не имеет.

В заключение, сделав предварительно краткий обзор основных схем зоогеографического районирования Талыша, попытаемся предложить вариант, который нам представляется наиболее отвечающим истинному положению дел.

Первое, что следует отметить, это давно назревшую необходимость унификации зоогеографических подразделений; ибо, с одной стороны, зоогеографическое подразделение - это реально существующая совокупность

живых организмов, размещенная на определенной территории. С другой стороны, каждый из исследователей, изучавший ту или иную частицу совокупности, приходит к иным выводам, нежели его коллега, посвятивший время изучению другой частицы этой же совокупности; причины этого отмечены в начале статьи. Это вступление в данном случае наполнено вполне конкретным содержанием: И.А.Долгушин (1957), изучая птиц, пришел к выводу, что за собственно Средиземноморьем следует сохранить зоогеографическое подразделение, но невысокого ранга, не выше провинции. Термин "средиземноморская подобласть" указанный автор из зоогеографического обихода предлагает исключить. Наш материал не позволяет нам согласиться с этим мнением, ибо не менее трети наших видов связано так или иначе с этой территорией; эта разница во взглядах в данном случае подтверждает своевременность постановки вопроса о необходимости попыток унифицировать зоогеографические подразделения на основании сопоставления сведений, полученных разными специалистами на разных группах живых организмов.

Второй вариант "большого" подразделения предлагают авторы "Геоботанического районирования СССР" (1947), относящие гирканскую провинцию к Азиатской пустынной области. Даже если принять правомерность выделения такой области, то к ней можно отнести лишь нагорно-степную часть Талыша (которая, кстати, не относится к собственно гирканской провинции). Ленкоранская низменность, а тем более зона горных лесов нацело лишены черт, позволяющих принять этот вывод.

Этот взгляд в некоторой мере связан с мнением Метью (**Matthew**, 1908, не предлагавшего, правда, конкретных названий биогеографических районов, но указывающего на наличие гипотетического аеиатского центра распространения, давшего одну ветвь на восток - к Америке, и две родственные ветви на запад - к Африке и Европе.

Таким образом, вслед за большинством исследователей, мы относим Талыш к средиземноморской подобласти Палеарктики.

М.А.Мензбир (1934) выделяет Кавказскую провинцию, не вдаваясь в этой работе в более детальное дробление.

Форкарт (**Forcart**, 1935), а вслед за ним Стармюльнер и Эдлауер (**Starmühlner und Edlauer**, 1957), также рассматривают весь Кавказ как провинцию, включающую в себя ряд подпровинций, в том числе и гирканскую. Такой подход, на наш взгляд, является формальным, ибо Кавказ, являясь самостоятельной геоморфологической единицей (или, по мнению других авторов, совокупностью нескольких очень близких единиц), достаточно разнороден в зоогеографическом отношении, чтобы признать существование на его территории нескольких самостоятельных провинций.

О.Ретовский (1899) был первым из малакологов, который высказал мысль о выделении Талыша в самостоятельную зоогеографическую единицу.

Герпетолог Н.И.Соболевский предлагает выделять западноазиатскую провинцию с тремя округами: восточно-закавказским, южнокаспийским и иранским. Эти округа в Талыше в этом случае будут представлены участками соответственно: восточно-закавказских степей, талышским и зувандским. Наши материалы не позволяют соединять фауну Зуванда и нижележащих частей в одно целое, и поэтому помещение этих территорий в пределы одной провинции нам не представляется правомерным. С более дробными подразделениями указанного исследователя нельзя не согласиться.

Наиболее обоснованную, с нашей точки зрения, схему, отвечающую последним данным, предлагает Н.Г.Самедов (1963). На территории Талыша он различает две провинции: Ирано-Азербайджанскую и Гирканскую. Нагорно-степная зона, в свете этих представлений, составляет Зувандский участок Мало-Кавказского округа первой из отмеченных провинций. Лесная зона (на низменности и восточных склонах) составляет Ленкоранский округ Гирканской провинции.

Небольшие несоответствия наших взглядов с приведенными выше заключаются в следующем. Мы не усматриваем существенной разницы между малакофауной Мало-Кавказского округа и прилежащих частей нагорного Ирана и не видим смысла поэтому в особом выделении такого округа. Выделение же Зувандского участка нам представляется оправданным в силу отсутствия на нем ряда видов, свойственных более южным частям этой территории (южнее богаче представлены **Enidae**).

Соглашаясь вполне с мнением И.М.Лихарева (1957, 1962), мы считаем более правильным выделение Армянской провинции, а не Ирано-Азербайджанской, как это предлагает Н.Г.Самедов, т.к. первое название более точно отражает распространение видов, относящихся к данной зоогеографической группировке.

Выделение же в пределах гирканской провинции ленкоранского округа диктуется некоторым несоответствием в видовом составе между советской и иранской частями этой провинции, которое выражается в том, что иранская территория лишена некоторых видов, обитающих севернее (сведения по иранской части провинции см. в работах Форкарта (**Forcart**, 1935) и Стармюльнера и Эдлауера(**Starmühlner und Edlauer**, 1957).

Исходя из всего сказанного, мы предлагаем следующую схему зоогеографического районирования Талыша:

П А Л Е А Р К Т И Ч Е С К А Я О Б Л А С Т Ь

СРЕДИЗЕМНОМОРСКАЯ ПОДОБЛАСТЬ

Г и р к а н с к а я п р о в и н ц и я

Ленкоранский округ

А р м я н о - И р а н с к а я п р о в и н ц и я

Зувандский округ

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ПРИЛОЖЕНИЕ

КРАТКИЕ ОПИСАНИЯ НОВЫХ ВИДОВ, ВКЛЮЧЕННЫХ В СТАТЬЮ.[4]

Семейство **Ellobiidae**

Carychium primitivum **Schileyko, sp.n.** Раковина удлиненно яйцевидная, со слегка заостренной вершиной. Отношение высоты раковины к ее ширине (В/Ш) - 1,9. Поверхность раковины крайне тонко радиально исчерчена. Оборотов 5, плавно нарастающих, довольно выпуклых. В устье три зуба: колумеллярная пластинка, базальный и палатальный бугорки. Высота устья составляет примерно одну треть высоты раковины. Колумеллярный край почти отвесный. Высота раковины 1,75 мм, ширина раковины 0, 75 мм. Устье: высота 0,65 мм, ширина 0,60 мм.

Колумеллярная пластинка похожа на таковую ***C. minimum*** **Müll.** - небольшая, край ее совершенно не образует изгибов (в сочетании с формой раковины - основной диагностический признак).

Семейство **Succineidae**

Succinoides **Schileyko, gen.n.** Имеется чехол пениса. Внутри пениса, близ места впадения семепровода имеется розеткообразная присоска. ***Succinoides stelliferus*** **Schileyko, sp.n.** Достоверных конхиологических отличий от ***Succinea sarsi*** **Esm. et Hayer** не найдено. Высота раковины 7,6 - 9,2 мм. Ширина раковины - 4,4 - 5,3. Высота устья - 4,4 - 5,5 мм. Ширина устья - 3,9 - 4,2 мм.

Гермафродитный проток образует небольшое число плотно навитых изгибов и петель. Характерно очень ранее разделение семепровода и яйцевода. Эпифаллус внешне не выражен.

Радула: $\frac{1с}{1} + \left(\frac{6б}{3} + \frac{36к}{2-3} \right) \times 2.$

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[4] Подробные описания будут опубликованы позже в друггих изданиях.

Семейство **Helicidae**

Theba longiflagellata **Schileyko, sp. n.** Проток семеприемника короткий; резервуар его не доходит до белковой железы. Половой ретрактор крепится к проксимальному концу пениса; длина бича составляет не менее половины суммарной длины пениса и эпифаллуса. Слизистых желез три.

$$\text{Радула: } \left(\frac{1с}{3} + \frac{15б}{2} + \frac{1к}{4} + \frac{16к}{2\text{-}5} \right) \times 2$$

Theba maxima **Schileyko, sp. n.** Проток семеприемника длинный, резервуар примыкает к белковой железе. Ретрактор пениса крепится близ границы пениса и эпифаллуса. Длина бича менее половины суммарной длины пениса и эпифаллуса. Слизистых желез две.

$$\text{Радула: } \left(\frac{1с}{3} + \frac{18б}{2} + \frac{11к}{3\text{-}4} \right) \times 2$$

Раковины этих двух видов похожи на раковину *Th. talyschana* (Mart.) и отличаются, главным образом, размерами. Половой аппарат, как было показано, демонстрирует существенные различия.

ABSTRACT

COMPOSITION AND PALEOGRAPHIC HISTORY OF THE TERRESTRIAL MOLLUSCAN FAUNA OF TALYSH, U.S.S.R.

A. A. Schileyko

The terrestrial mollusk fauna of Talysh (southeastern Transcaucasia, USSR) comprises 62 species which can be arranged zoogeographically into 8 groups: Holarctic, Palearctic, Mediterranean, Middle European, Middle Asiatic, Caucasian, Armenian-Iranian and endemic Hyrcanian. A detailed analysis of the ecological characteristics of the representatives of the first 2 groups leads to the conclusion that the settlement of most of these forms occurred relatively late.

In order to determine the time and possible routes of the penetration of mollusks, the paleography of the region has been reconstructed on the basis of published data, beginning with the time of its appearance as an island (Upper Pliocene) to the present. The following temporary possibilities of penetration of mollusks are apparent: there was an invaision from the Caucasus during the Middle Miocene to Upper Pliocene; from Middle Europe and Mediterranean during the Upper Miocene to the end of the Lower Pliocene; from Asia Minor during the Lower Miocene to Upper Pliocene; and from Eastern Asia during the Lower Miocene to Lower Pliocene. Thus, the high degree of isolation of the region during the entire time of its existence is apparent. In most recent times the territory of wooded Talysh has been completely isolated, with reference to its mollusk fauna, from the adjacent regions: from the east by the Caspian Sea, from the north by the expanse of semi-desert areas of Kura-Araxin lowlands, and from the south and west by arid regions of the woodless upland of Iran.

From an analysis of the composition of endemic species inference is made concerning their zoogeographic and genetic diversity. The Hyrcanian endemic species may be divided into 6 groups on the basis of their faunal relationships: south-Asiatic; tropical; middle European; Mediterranean; Caucasian; and minor-Asiatic.

From a comparison of qualitative composition of Talysh with that of other western Palearctic regions, it is concluded that the Talysh fauna is an impoverished one. The cause of this is apparently due to the unfavorable climatic conditions (a characteristic feature is a prolonged period of summer dryness lasting up to 120 days); weak calcium content of the soil; and, principally, the

isolation of the territory. The high percentage of endemic species (over 30%), the impoverished fauna, and certain one-sidedness of its development lead to the idea of the insular character of Talysh fauna. It may be considered that the present fauna of the wooded part of Talysh is an isolated community which, within a relatively brief period of time, was formed by zoogeographical influence and, to a small degree, was also influenced by conditions of a peculiar climate.

The necessity of unification of zoogeographical subdivisions by comparing information published by various authors about different groups of organisms inhabiting a given region is stressed. In conclusion, a critical analysis is given of different versions of zoogeographical subdivisions proposed in the literature, and a new version is suggested as the most logical according to the material collected by the author:

PALEARCTIC REGION
Mediterranean Subregion
Hyrcanian Province
Lencoran District
Armenian-Iranian Province
Zuwand District

RÉSUMÉ

CONSTITUTION ZOOGÉOGRAPHIQUE ET HISTOIRE DE LA FORMATION DE LA FAUNE MALACOLOGIQUE TERRESTRE DE TALYSH

A. A. Schileyko

La faune malacologique terrestre de Talysh (Sud-est de la Transcaucasie, U.R.S.S.) comprend 62 espèces, qui peuvent être classées en 8 groupes zoogéographiques: holarctique, paléarctique, méditerranéen, médio-européen, médio-asiatique, caucasien, armenio-iranien, et hyrcanien endémique. Une analyse détaillée des caractères écologiques des représentants des 2 premiers groupes conduit à conclure que la plupart de ces formes sont apparues relativement tard.

Dans le but de déterminer l'époque et les routes possibles de pénétration des mollusques, la paléontologie de la région a été reconstituée sur la base des publications, depuis l'époque où elle apparait comme une île (Pliocène supérieur) jusqu'à l'époque actuelle. Les possibilités de pénétration des mollusques paraissent être les suivantes: il y a eu une invasion d'origine caucasienne du Miocène moyen au Pliocène supérieur, d'origine médio-européenne et méditerranéenne du Miocène supérieur au Pliocène inférieur, d'Asie mineure du Miocène inférieur au Pliocène supérieur et d'Asie orientale du Miocène inférieur au Pliocène inférieur. Ainsi, le grand isolement de la région durant tout le temps de son existence est apparent. Dans les temps les plus récents, le territoire boisé de Talysh a été complètement isolé, si l'on considère la faune malacologique, des régions voisines: à l'est par la mer Caspienne, au nord par les étendues semi-désertiques des basses terres de Kura-Araxin et au sud et à l'ouest par la région aride des hautes terres non boisées de l'Iran.

D'après l'analyse de la composition des espèces endémiques, on en a déduit leur diversité zoogéographique et génétique. Les espèces endémiques hyrcaniennes peuvent être divisées en 6 groupes d'après leurs relations faunistiques: sud-asiatique, tropical, médio-européen, méditerranéen, caucasien et d'Asie mineure.

D'après une comparaison de la composition qualitative de Talysh avec les autres régions paléarctiques occidentales on arrive à la conclusion que la faune de Talysh est appauvrie. La cause en est apparemment due à des conditions climatiques défavorables (un des faits caractéristiques est la période de sécheresse estivale qui peut atteindre 120 jours); au peu de calcium contenu dans le sol, et, surtout, à l'isolement du territoire. Le fort pourcentage d'espèces endèmiques (plus de 30%), la faune appauvrie et une certaine unilatéralité de son développement, conduisent à l'idée d'un caractère insulaire de la faune de Talysh. On doit considérer que la faune actuelle de la partie boisée de Talysh est une communauté isolée qui, dans les limites d'une période relativement brève, a été constituée sous des influences zoogéographiques et, à un degré moindre, sous l'influence d'un climat particulier.

On souligne la nécessité d'unifier les subdivisions zoogéographiques, en comparant les informations publiées par les différents auteurs sur les divers groupes habitant une région donnée. En conclusion, il est donné une analyse critique des différentes

versions de subdivisions zoogéographiques proposées dans la littérature, et une nouvelle version est suggerée comme étant la plus logique compte tenu du matériel collecté par l'auteur.

REGION PALEARCTIQUE
Subrégion Méditerranéenne
Province hyrcanienne
District Lencoran
Province armeno-iranienne
District Zuwand

RESUMEN

COMPOSICION E HISTORIA PALEOGEOGRAFICA DE LA MALACOFAUNA TERRESTRE DE TALYSH, U. R. S. S.

A. A. Schileyko

La fauna de moluscos terrestres de Talysh (sureste de Transcaucasia, U. R. S. S.), comprende 62 species que pueden distribuirse zoogeográficamente en 8 grupos: Holártico, Paleártico, Mediterráneo, Central-Europeo, Central-Asiático, Caucasiano, Armenio-Iraniano y endémico Hyrcaniano. Un análisis detallado de las caracteristicas ecológicas de los representantes de los dos primeros grupos, nos lleva a la conclusión de que su colonización ocurrió relativamente tarde.

Con el objeto de determinar la época y las posibles rutas de penetración, la paleogeografía de la región ha sido reconstruida en base a datos ya publicados, comenzando desde el tiempo de su aparición como una isla (Plioceno Superior) hasta el presente. Las posibilidades temporarias de penetración fueron las siguientes: hubo una invasión desde el Cáucaso durante el Mioceno Medio, a Plioceneo Superior; del centro de Europa y del Mediterraneo desde el Mioceno Superior hasta el Plioceno Inferior; desde el Asia Menor durante el Mioceno Superior hasta el Plioceno Inferior; y del Asia oriental durante el Mioceno Inferior al Plioceno Inferior. Así, el alto grado de aislamiento de la región, durante todo el tiempo de su existencia, es aparente, En tiempos más recientes, el territorio boscoso de Talysh fue completamente aislado, en lo que a su malacofauna se refiere, de las regiones adyacentes: al oeste por el Mar Caspio, al norte por las extensas áreas semidesiertas de los bajos de Kura-Araxin, y al sur y oeste por las regiones áridas de la meseta de Iran.

Por un análisis de la composición de las especies endémicas se puede inferir su diversidad zoogeográfica y genética. Las especies endémicas Hircanicas pueden dividirse en 6 grupos sobre la base de sus relaciones faunisticas: Sud-Asiáticas; tropicales; central-Europeas; Mediterráneas; Caucásicas; y del Asia Menor.

Una comparación cualitativa de la composición de Talysh con otras regiones occidentales paleárticas, demuestra que su fauna se ha empobrecido. Las causas aparentes de esto son las desfavorables condiciones climáticas (un aspecto caracteristico son los largos veranos, con sequías de 120 dias de duración), el débil contenido de calcio en el suelo, y principalmente, el aislamiento territorial. El alto porcentaje de especies endémicas (mas del 30%), la fauna empobrecida, y cierta dirección única en su desarrollo, dan la idea del caracter insular de la fauna de Talysh. Puede considerarse que la fauna actual de la parte boscosa es una comunidad aislada, la cual, en un periodo relativamente breve, se formó por la influencia zoogeográfica de su origen, y en menor grado influenciada también por las condiciones climáticas.

Se particulariza la necesidad de unificar divisiones zoogeográficas, comparando informaciones publicadas por varios autores sobre grupos diferentes de organismos habitando una misma región. En conclusión se da un análisis crítico de las diferentes versiones sobre divisiones zoogeográficas propuestas en la literatura, y se sugiere una nueva interpretación, como la más lógica de acuerdo al material colectado por el autor:

Region Paleártica
Subregión Mediterránea
Provincia Hyrcaniana
Distrito Lencorano
Provincia Armenio-Irania
Distrito Zuwandano

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NOTES ON SEMAI ETHNOMALACOLOGY

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ABSTRACT

This paper presents some Malayan malacological data from the viewpoint of an anthropologist, i.e., in terms of the way aboriginal people think about and use mollusks. Among the Semai, an inland people whose diet is rather low in protein, mollusks serve primarily as food, also as a source of lime to chew with betel nut and, less often, as ornaments. Ritual restrictions govern their consumption. The Semai do not classify mollusks into a single category. Freshwater mollusks (*Melanoides, Pila*) can be eaten together with other "water creatures" (*ka'*) but not with any of the other 3 main categories of food (land vertebrates, birds and fungi). Land snails, which are not eaten, constitute the category *"too'."* The marine bivalve *Anadara* (*Arca*), a recently introduced "market food," is not subject to any taboo.

It is hoped that this sort of paper will lead to a more fruitful exchange between anthropologists and malacologists.

INTRODUCTION

This paper is a brief account of the ways in which a specific Austroasiatic-speaking aboriginal people, the Semai, who live in the center of the Malay peninsula, use mollusks and talk about them. The term "ethnomalacology" is slightly facetious, since the prefix "ethno-" has been rather lavishly applied to any and all systems of folk classification (see Sturtevant, 1964: 99-100). The general assumption implied by this prefix is that the data will be presented in terms of the classification used by the people studied rather than in terms of some European classification like Linnean binomials. The reason for such a presentation is that people respond to their environment as they classify and conceive of it, not the way an outsider conceives of it.

There are 3 reasons for presenting these data to an audience of malacologists. The first is that malacologists sometimes fail to realize that their work can be relevant to anthropology. They therefore usually neglect to gather information that would be of anthropological significance. Similarly, because anthropologists tend to be ignorant of malacology, they know relatively little about the relationships between non-industrialized peoples and mollusks, despite such studies as Harrington's (1945) on the use of mollusks by American Indians. Second, in a society where increasing specialization sometimes gives a scientist the feeling of being a master of arcana, it may be refreshing for him to know that a group of aborigines in the Malayan rainforest share his concern with facts and their categorization. Finally, the knowledge of how a people of a totally different background deal with one's own subject matter not only helps to clarify one's own ideas by contrast but also provides a framework for the understanding of the philosophy (or psychology) of science.

The data here presented were collected in 1962-63, during which time

the author and his wife lived for 7 months in each of 2 Semai villages, supported by a Ford Foundation grant. Settlement A was far inland, relatively isolated from the coast. Settlement B was in the coastal State of Perak and had access to marine products by way of the local market. The location of these settlements and others referred to in the text is given in Fig. 1.

The position of the Semai is rather like that of American Indians, except that the Semai have been able to preserve their way of life relatively intact. Their economy is based on slash-and-burn agriculture, with supplementary hunting and fishing. There is no significant political integration above the village level, although Settlement B shows signs of acculturation to the culture of the surrounding Malays.

Fig. 1. Malay Peninsula. Solid line surrounds Semai area.
1. Settlement A
2. Settlement B
3. Raub
4. Ulu Bertam

SEMAI MALACOLOGY

The Semai accord ritual signficance to 4 categories of organisms: *mənhar*, *cheb*, *bətiis* and *ka'*.[1] The first category comprises land vertebrates, the second refers to birds and the third to fungi. In the original usage, the term *ka'* refers generally to water creatures, exclusive of insects. It is taboo (*pənali'*) to prepare a meal that includes organisms from more than one of these categories. With some minor exceptions, this taboo does not apply to other organisms.[2]

Apparently the Semai do not conceive of mollusks as constituting a single taxonomic unit.[3] In Settlement A aquatic mollusks fall into the category *ka'*, a term which also applies to fish, crabs, amphibians and shrimp. As *ka'*, one may cook and eat them mixed together with other *ka'*. Cooking or eating them together with the other 3 categories of food, however, is taboo, seemingly because it would be a violation of the metaphysical, quadripartite division of the biological world. Settlement B people use the term *ka'* for fish only, presumably in imitation of the way the Malays use the equivalent word *ikan*. Nevertheless, the people in Settlement B continue to keep aquatic mollusks isolated from the other 3 categories of food and to mix them with fish. One might say that the category "water creatures" is marked linguistically and behaviorally in Settlement A but only behaviorally in Settlement B.

Too' is the category that embraces

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[1]The inverted e stands for a "schwa," an unaccented vowel like the u in circus or o in atom. The apostrophe stands for a glottal stop.

[2]For a more extended account of this taboo system, see Dentan, 1968a: 34-37.

[3]Neither do American Indians, according to Harrington, 1945.

land snails and no other organisms. They are not considered edible. There seems to be no other more general category which embraces *too'* or is coordinate with Settlement A's *ka'*. The closest the Semai seem to come to such a category is "those little things," a phrase used sometimes also for insects (Dentan, 1968b). This phrase is usually used dismissively, e.g., "Who knows anything about those little things?"

ANATOMY

All edible mollusks are said to smell *pəl'inh*, "like a knife being sharpened." The shell is the husk (*ho'*), the lip is the "lip," the inner chamber is the "room," and the soft part of the organism is the "insides." The operculum is the "lid" (*jərənəkeb*, lit., "that which closes") or the "tongue." The eyestalks are the "horns" or "mustache."

UTILITY

The Semai use mollusks in 3 ways: as ornaments, as food and as sources of lime for chewing. These uses are summarized in Table 1. Shell ornaments -- mostly necklaces, pendants and bracelets (see Fig. 2) -- have largely gone out of style. Based on an examination of early photographs of the Semai, my impression is that shell ornaments were never so popular as to have any significant impact on the mollusk population. Shells are still sometimes used as ornamental toggles for dart quivers.

The Semai do not eat any of the land mollusks listed in Table 1, i.e., among others, the cyclophorid mesogastropods *Pollicaria* and *Cyclophorus* and the stylommatophoran pulmonates *Amphidromus* and *Achatina*. Eating one, informants said, would cause "retching."

The Semai usually stew or boil the aquatic mollusks with spices such as hot peppers, salt, onions, sometimes mixing them with vegetables (e.g., tapioca, cucumber) or other aquatic creatures. Despite Medway's (1960: 377) remark that *Melanoides* is "rarely large enough to be worth eating," it is by far the most important mollusk in Settlement A, both as a source of food and also as a source of lime. There seem to be 2 main reasons for its relative importance as a foodstuff. This group of Semai is hard-pressed to get protein. They feel that a meal without meat or at least *ka'* is "not really eating." As a result they will collect and eat very small animals (e.g., grasshoppers or small guppies), provided that (1) the

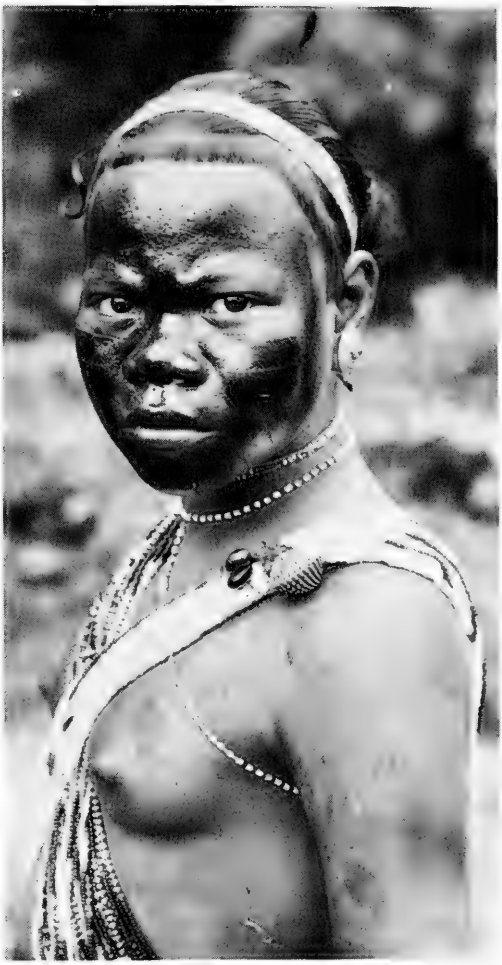

FIG. 2. Semai girl with *Anadara* shell ornament (probably from near Raub, 1930; photograph by courtesy of Mr. Louis Carrard).

TABLE 1. Use of mollusks among aborigines (Semai) in the Malay Peninsula

SOURCE	TAXONOMY		FOOD			OTHER USES	
	Semai	Linnean	Eaten	Taboo during Pregnancy	Taboo Postpartum	Lime	Ornament
Settlement A	*Ka'Kob*[1]	*Melanoides variabilis*[2]	Yes	Womb closes like operculum	Smell causes malaria	Yes	?No
	Too'	*Amphidromus*[2]	No	Not applicable	Not applicable	No	Yes
	Too' waal[3]	unidentified	No	Not applicable	Not applicable	No	Yes
Settlement B	*Kalo'*	*Melanoides variabilis*	Yes	No	For 44 days; smell upsets womb	Yes	No
	Kəmbuei[4]	*Pila scutata*	Yes	No	No	Rarely	No
		P. ampullacea	Yes	No	No	Rarely	No
	Too'	*Achatina fulica*	No	Not applicable	Not applicable	No	No
	Kəreg[4]	*Anadara granosa*	Yes	No	No	Yes	Yes[5]
Raub[6]		"*Ampullarius*" (=*Pila*)	Yes				
?Ulu Bertam		*Pollicaria elephas*					Yes[7]
"Perak Sakai"[8]		"*Bulimus*" (=*Bithynia*)					Yes
		"*Hybocystis*" (=*Pollicaria*)					Yes
		Helix					No
		Cyclophorus					No

[1]Literally "lime aquatic-creature."

[2]Identified by Zoology Department, University of Malaysia.

[3]Lit. "fireplace land-snail." Semai hearths are circular and flat, like the snail.

[4]Name is of Malay origin.

[5]Pendant illustrated in Williams-Hunt (1952): Pl. 6d. Such ornaments, probably from the area around Settlement B, collected in late 1920's are at the Musée d'Homme (Paris), accession numbers 30. 54. 1606 and 30. 54. 1609. A necklace of these arc shells, collected by L. Wray in 1904 from a people related to the Semai, is in the Tapah Museum, accession number 103/04.

[6]Data from Burkill, 1935: 142.

[7]Specimen of necklace on exhibit, Field Museum of Natural History (Chicago), accession number 46722, collected in 1920's, identified by Dr. Alan Solem. Probably other *Pollicaria* spp. were used the same way.

[8]Skeat and Blagden (1906, I: 145, 152). The "Perak Sakai" are either western Semai or Temiar, a group closely related to the Semai. The authors unfortunately do not specify the location. There is, however, no reason to think that the Temiar use mollusks any differently from the Semai.

FIG. 3. Preparing to make lime (Settlement A, 1962).

FIG. 4. Pyre ready to receive shells (Settlement A, 1962).

animals occur in large numbers in a small area or (2) that they can be collected effortlessly during the course of another activity. *Melanoides* meets both these conditions. It is very common in small streams; and it can be collected while one is fishing, walking home from one's fields or even defecating in the stream. The only difficulty with eating *Melanoides*, as Medway (1960: 370) notes, is that the shell is a sharply elongated spiral, so that people must smash the apex before sucking or tapping out the meat.

The most important food mollusk in Settlement B is the protein-rich marine bivalve, *Anadara* (=*Arca*) *granosa*. This clam is a common food of poor people and thus of acculturated aborigines throughout Malaya. Perhaps because it is a fairly recently introduced food, people say that no taboos govern its preparation or consumption. Indeed, some Semai say that, since it is a "market" rather than a "water" food, it is not subject even to *pənali'* rules.

In both settlements men and women make lime for chewing the betel nut. *Melanoides* is the traditional source of lime, but *Anadara* is replacing it in Settlement B. There is no ritual involved, and each individual makes his own lime. The limemaker splits dry

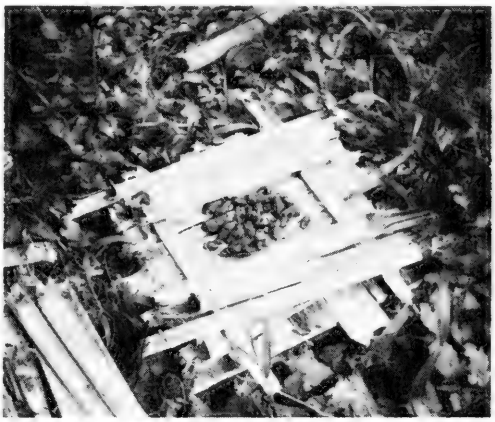

FIG. 5. *Melanoides* shells in position for burning (Settlement A, 1962).

bamboo longitudinally into kindling. He takes the kindling outdoors and lays half a dozen pieces parallel to each other on the ground. He then lays another layer of kindling on top of the first but at right angles to it. This process continues until the pile is about 6 layers deep (see Figs. 3 and 4). The shells are put in a sort of nest on top of the sixth layer (see Fig. 5), and another 5 or 6 layers are added to the pile of kindling.

After the shells are burnt, the limemaker scoops them up in half a length-

wise-split bamboo internode and takes them home. There he picks out the relatively undamaged shells, blows on them to remove impurities and stores them away in an unornamented bamboo internode for possible future burning. He and those of his housemates who feel like helping him out then take small portions of the remaining mixture of ash and line into their cupped hands. They then try to blow the ash away, leaving the lime. Some lime is lost this way, but apparently most of the impurities are winnowed out.

The only other mollusk of much economic significance to the Semai is the giant African land snail (*Achatina*), which was introduced into Malaya about 1922 (Burkill, 1935: 28). Although it has not yet penetrated far enough inland to be a pest at Settlement A, it is very common at Settlement B and does such damage to house gardens that people have almost given up planting crops around their houses. For some reason it appears to be much less common in the fields that are located some distance away from the settlement, conceivably because the fields are at a higher altitude and relatively isolated from each other. The Semai recognize the harm this snail does, but they make no attempt to control it. They say it is "not pretty" and therefore no good for making ornaments. Moreover, they insist (wrongly, I think) that it cannot be used for food or lime. Finally, they have no specific name for it, referring to it merely as *too'*, the generic term for land snail. It may be that the recency of the animal's introduction accounts for the Semai failure to come to grips with this new element in their environment.

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RÉSUMÉ

NOTES SUR L'ETHNOMALACOLOGIE DES SEMAÏ

R. K. Dentan

Cet article présente quelques observations de malacologie malaise faites par un anthropologiste qui étudie la façon dont les aborigènes considèrent et utilisent les mollusques. Chez les Semaï, une peuplade de l'intérieur dont le régime est assez pauvre en protéines, les mollusques servent avant tout de nourriture, mais aussi de source de chaux pour les chiques de bêtel et, plus rarement, d'ornements. Des restrictions rituelles codifient leur consommation. Les Semaï ne classent pas les mollusques en une seule catégorie. Les mollusques d'eau douce (*Melanoïdes*, *Pila*) peuvent être mangés en même temps que les autres "créatures aquatiques" (*ka'*)

mais pas avec les trois autres principales catégories d'aliments (vertébrés terrestres, oiseaux et champignons). Les testacés terrestres, qui ne sont pas consommés, constituent la catégorie *"too'."* Le bivalve marin *Anadera (Arca)*, une "denrée commerciale" d'introduction récente, n'est l'objet d'aucun tabou.

On espère qu'un article de cette nature conduira à des échanges plus fructueux entre anthropologistes et malacologistes.

RESUMEN

NOTAS SOBRE ETNO-MALACOLOGIA

R. W. Dentan

Este trabajo presenta algunos datos sobre malacología malaya, desde el punto de vista del antropólogo, esto es, en términos de como los aborígenes consideran y utilizan los moluscos. Entre los Semai, un pueblo del interior, cuya dieta es más bien pobre en proteínas, los moluscos sirven primeramente como alimento y también como elemento calcáreo para masticar con la nuez betel; con menos frecuencia se usan como ornamento. El consumo es gobernado por restricciones rituales. Los Semais no distinguen los moluscos como una categoría única. Los dulceacuícolas (*Melanoides, Pila*) pueden comerse con otras "criaturas de agua dulce" (*Ka'*) pero no con ninguna de las otras tres categorias de alimento (vertebrados terrestres, aves y hongos). Caracoles terrestres, que no son consumidos, constituyen la categoría *"too."* El bivalvo marino *Anadara (Arca)*, introducido recientemente como "alimento de mercado" no está sujeto a ningun tabú.

Es de esperar que este tipo de trabajo conduzca a un provechoso intercambio de ideas entre antropólogos y malacólogos.

АБСТРАКТ

ЗАМЕТКИ ПО ЭТНО-МАЛАКОЛОГИИ НАРОДНОСТИ СИМЭЙ

Р. К. ДИНТЕН

Настоящая статья рассматривает некоторые данные по малайской малакологии с точки зрения антрополога т. е. на основе выражений, при помощи которых местные аборигены думают и говорят о моллюсках, которых они употребляют.

Среди Симэй, народности, населяющей внутреннюю часть страны, пища которых довольно бедна белками, моллюски служат прежде всего пищей, а также и источником извести, необходимой при жевании бетеля, реже - как украшения. Ритуальные ограничения определют степень использования ими моллюсков. Симэй не считают всех моллюсков одинаковыми. Пресноводные моллюски (*Melanoides, Pila*) могут употребляться в пищу вместе с другими "водными животными" (ка'), но ни с одной из трех других главных пищевых групп (наземных позвоночных, птиц, грибов). Наземные улитки, которых не употребляют в пищу, составляют группу "туу". Морские двустворчатые моллюски *Anadara (Arca)*, недавно интродуцированные сюда как рыночный пищевой объект, не служат объектом какого-нибудь "табу".

Автор надеется, что статьи, подобные настоящей, приведут к плодотворному обмену мнениями между антропологами и малакологами.

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CYTOTAXONOMIC STUDIES OF LYMNAEID SNAILS[1]

Akihiko Inaba[2]

ABSTRACT

The chromosomes of 16 species of lymnaeid snails from 22 localities were observed during spermatogenesis, as well as during oögenesis and mitotic divisions. The chromosomes of 7 species and subspecies are reported for the first time in this paper. In the other species new knowledge of chromosomes in spermatogonia, oöcytes, polar bodies and somatic mitoses is added to previous reports. The chromosome numbers determined are compared with those previously reported. In the Lymnaeidae, chromosome numbers now have been reported for 41 species and subspecies belonging to 7 genera. Based on cytological features, various taxonomic points are discussed in each group with different chromosome numbers.

Lymnaea (4 subspecies), *Stagnicola* (15 species and subspecies), *Acella* (1 species), *Pseudosuccinea* (1 species) and *Bulimnea* (1 species) all have the haploid chromosome number 18. No obvious morphological difference or remarkable characteristics were found to distinguish caryotypes among or within these genera. Three species of *Fossaria* have the haploid number 18, but *F. rustica* has 19. This suggests that the latter should be raised to the rank of species, although many authors in the past considered this snail as a form or subspecies of *F. modicella*. The additional chromosome pair is small and often rather weakly staining during spermatocyte diakinesis.

Radix (11 species and subspecies) has 17 haploid chromosomes. *"Lymnaea" natalensis* from Liberia also has n=17, which suggests that it is a *Radix* species. Anatomical features confirm this. Three lymnaeid species have only 16(n) chromosomes: *"L." ollula* (=*viridis*?), *"L." tomentosa* and *"L." lessoni*. They have been included in various nominal generic groups, mainly by their conchological features. However, a new group name may be needed for those 16(n) species.

Somatic chromosomes were observed in 5 species (*Stagnicola palustris wyomingensis, S. exilis, S. catascopium, Bulimnea megasoma* and *Fossaria rustica*). Generally, all chromosomes in somatic mitoses of young embryos were meta- or submetacentric in nature. These chromosomes may be very useful for caryotype analyses of lynmaeid snails, but more detailed observations are needed before reliable conclusions can be drawn from comparative studies.

A phylogenetic consideration of Lymnaeidae based on cytotaxonomic, cytological and paleontological information is presented.

INTRODUCTION

The basommatophoran Lymnaeidae are one of the most extensively studied groups of mollusks in regard to their chromosome numbers. Nevertheless, only a few caryotype studies have been undertaken on this family. Perrot &

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Perrot (1938) studied the chromosome numbers in 6 species of lymnaeid snails and postulated that 3 subgenera (*Radix*, *Stagnicola* and *Lymnaea* s.s.) should be grouped into 2: *Radix* (n=17) and *Stagnicola-Lymnaea* (n=18); but this has not been widely accepted among taxonomists. Inaba & Tanaka (1953) reported the chromosome numbers of 2 lymnaeid snails in Japan, but recently Burch, et al. (1964) and the author (1965) corrected these earlier chromosome reports. Natarajan (1960) reported on the chromosomes of a species of *Radix* from India.

Burch (1960a) reported on the shapes of mitotic chromosomes in aquatic pulmonate snails which included 2 lymnaeids, and, more extensively, he (1960b) studied 18 species and subspecies of the Lymnaeidae. Burch, et al. (1964) studied the chromosomes of 3 *Radix* species and a *Fossaria* from Japan. They found the lowest chromosome number of lymnaeid snails in *"Lymnaea" ollula* (n=16), and suggested that this species should be placed in the genus *Radix* because of its cytological features, instead of the genus *Fossaria*, where it had been previously placed. Burch (1965) presented the possible relationships of various genera of the Lymnaeidae, based on their chromosome numbers, in his general review of cytotaxonomy in euthyneuran snails.

MATERIALS AND METHODS

Sixteen species and subspecies of lymnaeid snails from 22 localities were used in this investigation. The chromosomes in 9 of these species were reported previously by Burch (1960a,b). A list follows to indicate the species studied here as well as to give exact localities for obtaining the snails. An asterisk (*) indicates the species previously reported, and a double asterisk (**) indicates that the specimens were from the same locality as those studied by Burch (1960a, b). Shells of specimens from populations of the current study are shown in Figs. 83-102.

List of species and localities

Stagnicola palustris wyomingensis Baker, 1927. Giggey Lake, Boulder Co., Colorado, U.S.A., by George W. Bryce, June 25, 1967; "Lodge of the Pines," Boulder Co., Colorado, by George W. Bryce, June 25, 1967.

**Stagnicola umbrosa* (Say, 1832). **Roadside pond, 3 miles Northeast of East Tawas on US 23, Iosco Co., Michigan, U.S.A., May 18, 1967.

**Stagnicola exilis* (Lea, 1837). Small pond at junction of Dancer Rd. and Trinkle Rd. near Dexter, Sec. 11, Lima Township, Washtenaw Co., Michigan, U.S.A., by J. B. Burch, May 2, 1967; Small pond at junction of Dancer Rd. and Jackson Rd., Sec. 15, Lima Township, Washtenaw Co., Michigan, by J. B. Burch, May 10, 1967.

**Stagnicola catascopium* (Say, 1817). **Au Sable River, public fishing site, just off highway M-72, 4 miles east of US 27, near Grayling, Crawford Co., Michigan, U.S.A., May 18, 1967; Beach pool at Hammond Bay, Presque Isle Co., Michigan, by R. H. Russell, Sept. 4, 1967.

**Stagnicola emarginata serrata* (Haldeman, 1842). Southwest side of Higgins Lake, Roscommon Co., Michigan, U.S.A., May 18, 1967.

Stagnicola hinkleyi (Baker, 1906). NW¼, NW ¼, Sec. 26, T. 39 N., R. 116 W., Snake River, Teton Co., Wyoming, U.S.A., by D. W. Taylor, Aug. 29, 1959.

Stagnicola idahoensis (Henderson, 1931). NW ¼, Sec. 25, T. 21 N., R. 1 E., Little Salmon River, Idaho Co., Idaho, U.S.A., by D. W. Taylor, Sept. 29, 1959.

Stagnicola cf. *bonnevillensis* (Call, 1884). SW ¼, Sec. 36, T. 42 N., R. 116 W., Spring at base of Cobble gravel terrace, Teton Co., Wyoming, U.S.A., by D. W. Taylor and J. D. Love, Aug. 24, 1959.

**Pseudosuccinea columella* (Say, 1817). Bass Lake, Unadilla Township, Livingston Co., Michigan, U.S.A., by J. B. Burch, May 1, 1967.

**Bulimnea megasoma* (Say, 1824). **Roadside drainage ditch, highway

M-55, about 2 miles West of Houghton Lake, Roscommon Co., Michigan, U.S.A., May 18, 1967.

**Fossaria parva* (Lea, 1841). Small roadside drainage ditch, 1 mile West of Clio, Genesee Co., Michigan, U.S.A., by R. H. Russell, July 15, 1967.

**Fossaria modicella* (Say, 1825). Fleming Creek, at Parker's Mill, 2 miles East of Ann Arbor, Washtenaw Co., Michigan, U.S.A., by J. B. Burch, May 9, 1967; Burnt Cabin Point, Huron Co., Michigan, by J. B. Burch, June 27, 1967; Comstock, Kalamazoo Co., Michigan, , by J. B. Burch, July 7, 1967.

**Fossaria rustica* (Lea, 1841). **Mill pond on highway M-132, Sec. 5, Scio Township, Dexter, Washtenaw Co., Michigan, U.S.A., Aug. 16, 1967; Pond, Willys Park, Toledo, Ohio, U.S.A., by R. H. Russell, Sept. 4, 1967.

Radix natalensis (Krauss, 1848). Liberia, West Africa, by Dr. Z. H. Abedi, Sept. 4, 1963. University of Michigan Laboratory stock.

"Lymnaea" tomentosa (Pfeiffer, 1855). South Australia, by Dr. J. C. Boray; received Feb. 13, 1967. University of Michigan laboratory stock.

"Lymnaea" lessoni (Deshayes, 1831). Small stream, 3 miles from Popondetta on Oro Bay Road, Papua, by J. B. Burch, Sept. 7, 1966.

The materials examined consisted of 1) ovotestes in active stages of gametogenesis and 2) egg masses within 2 or 3 days after spawning. Ovotestes dissected from living material were fixed in Newcomer's (1953) fluid and stained by the acetic-orcein squash technique (La Cour, 1941). A few species collected from Papua, South Pacific, Idaho and Wyoming (U.S.A.) were fixed and preserved in Newcomer's fixative for 1-9 years. Their ovotestes were also examined by squash preparations. In those species that chromosomes were studied from egg masses, the living embryos were taken out of the egg mass, and after breaking their individual capsules, the embryos were stained on a microscope slide with acetic-orcein either directly or after fixation in Newcomer's fluid. Observations were made with a Nikon microscope using a 100× immersion objective, 15× oculars, and achromatic condensor (n.a. 1.25). The chromosomes were drawn with the aid of a camera lucida and reproduced at a table-top magnification of 3,200×.

OBSERVATIONS

Chromosome numbers determined in this investigation are given in Table 1.

I. Chromosomes in spermatogensis.

1. *Stagnicola*

Eight species collected from 11 localities were investigated. All of the species of *Stagnicola* observed in this study have the same chromosome number (n=18), the same number found for this genus in previous reports. There seems to be very little difference between *Stagnicola palustris wyomingensis* (Figs. 1-4) and 3 subspecies of *S. palustris* observed by Burch (1960b).

Stagnicola umbrosa (Figs. 5-7) and *S. catascopium* (Figs. 10, 11), collected from the same localities as Burch (1960b), and *S. exilis* (Figs. 8, 9), *S. catascopium* (Figs. 12, 13) and *S. emarginata serrata* (Figs. 14, 15), collected from different localities, also show the same appearance in meiosis as Burch (1960a,b) reported. The chromosome figures of *S. hinkleyi* (Figs. 16, 17), and *S.* sp. (cf. *bonnevillensis*) (Fig. 20) are also similar in appearance to the other *Stagnicola* species. In gonial cells of *S. umbrosa* (Fig. 5), *S. catascopium* (Fig. 12), *S. emarginata serrata* (Fig. 14), *S. idahoensis* (Fig. 18) and *S.* cf. *bonnevillensis* (Fig. 19), 36 meta- or submetacentric chromosomes were observed. No remarkable differences were found between them.

2. *Pseudosuccinea*

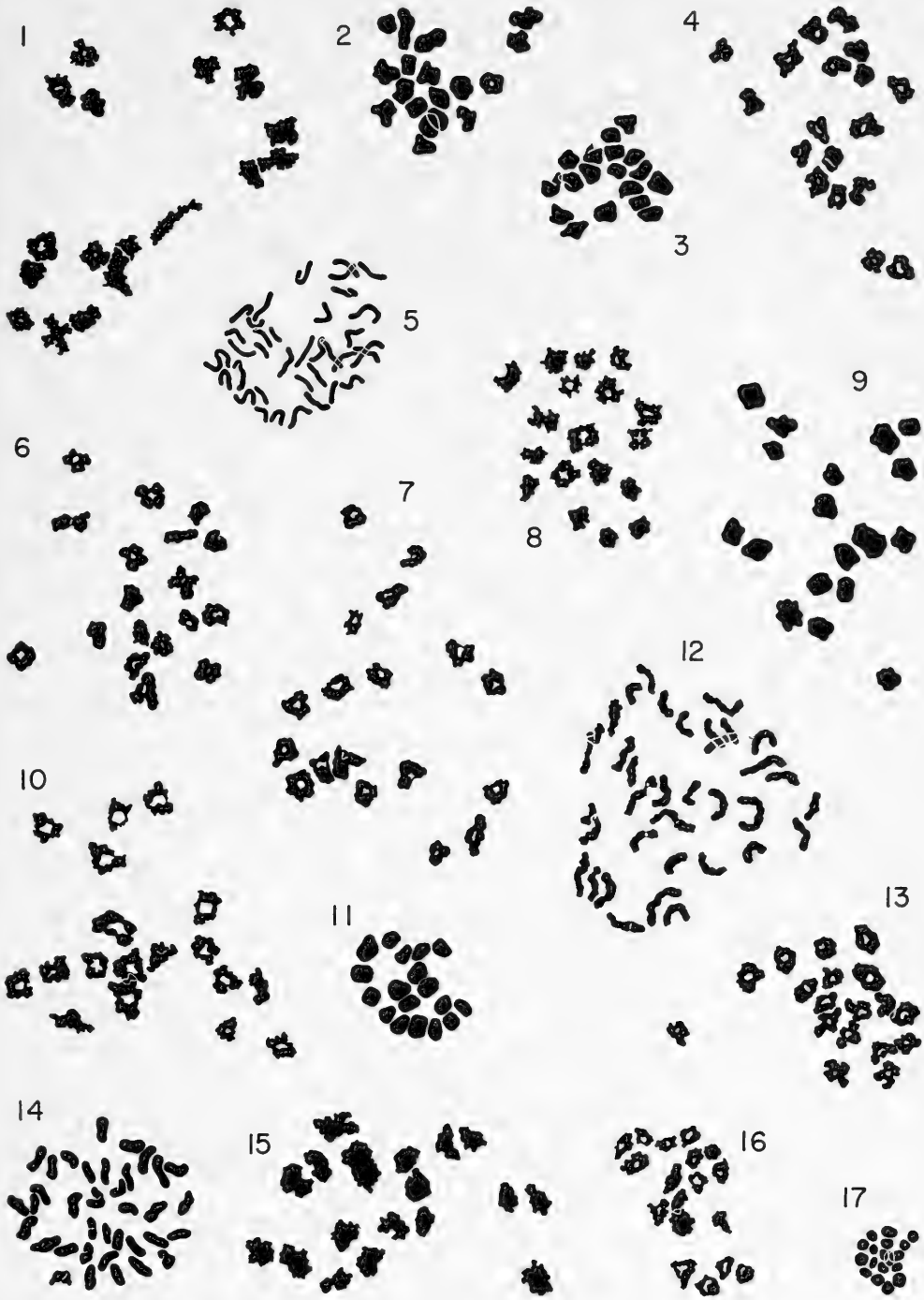
The caryotype of *Pseudosuccinea columella* from Michigan (Figs. 21, 22)

TABLE 1. The chromosome numbers determined in this investigation

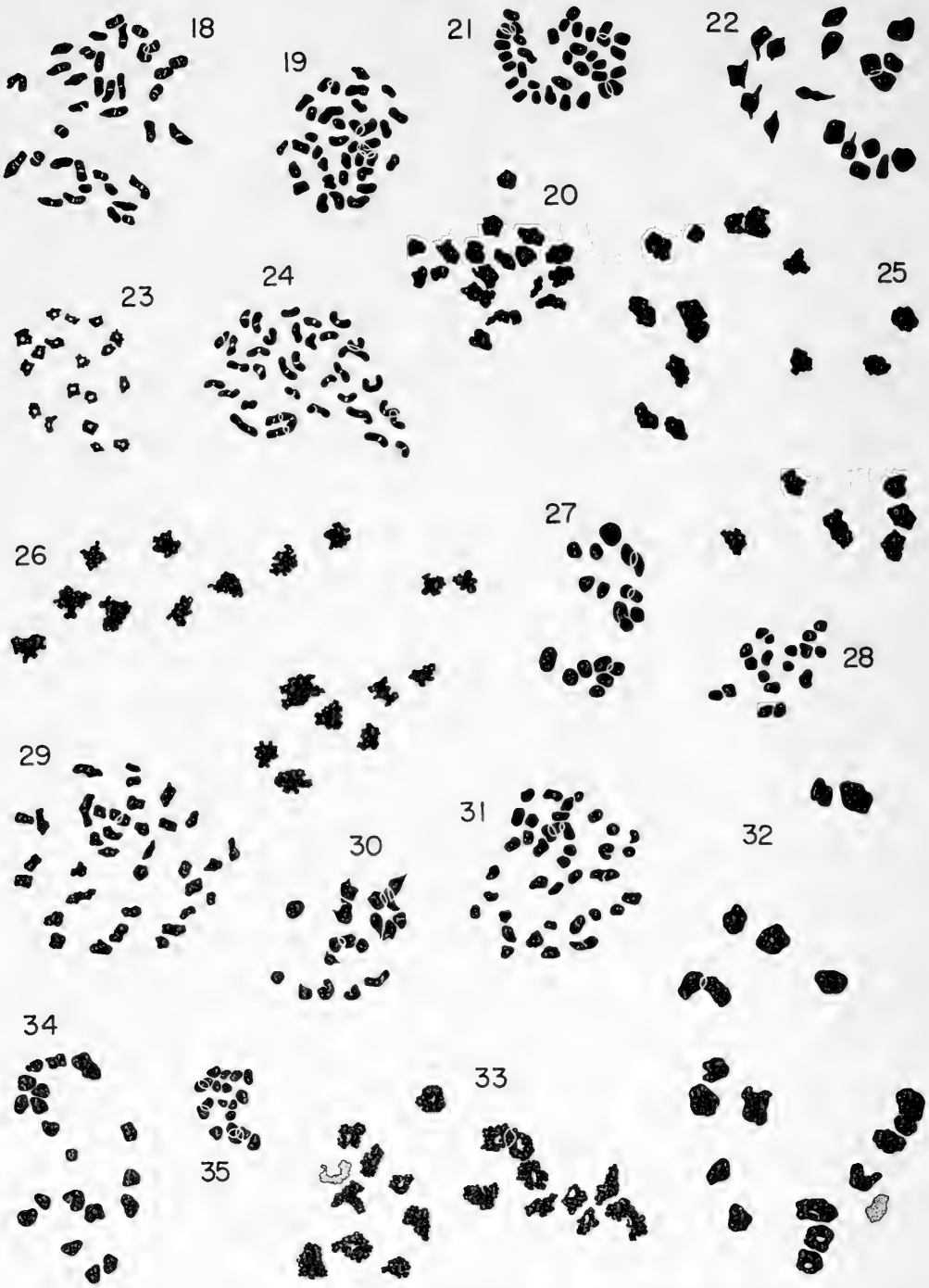
Species	2n		n			Locality
	spg	som	spc	oc	pb	
Stagnicola palustris wyomingensis	–	36	18(I)	–	18	Giggey Lake, Boulder Co., Colo.
"	–	–	18(I)	18	18	"Lodge of the Pines," Boulder Co., Colo.
S. umbrosa	36	–	18(I)	–	–	*Roadside pond, Iosco Co., Mich.
S. exilis	–	36	18(I)	18	–	Small pond, Dancer & Trinkle Rds., Washtenaw Co., Mich.
"	–	36	18(I)	–	18	Small pond, Dancer & Jackson Rds., Washtenaw Co., Mich.
S. catascopium	–	36	18(I)	–	–	*Au Sable R., Crawford Co., Mich.
"	36	–	18(I)	–	–	Beach pool at Hammond Bay, Presque Isle Co., Mich.
S. emarginata serrata	36	–	18(I)	–	–	Higgins Lake, Roscommon Co., Mich.
S. hinkleyi	–	–	18(I, II)	–	–	Snake River, Teton Co., Wyo.
S. idahoensis	36	–	–	–	–	Little Salmon R., Idaho Co., Ida.
S. cf. *bonnevillensis*	36	–	18(I)	–	–	Spring at base of Cobble gravel terrace, Teton Co., Wyo.
Pseudosuccinea columella	36	–	18(I)	–	–	Bass Lake, Livingston Co., Mich.
Bulimnea megasoma	–	36	–	–	–	*West of Houghton Lake, Roscommon Co., Mich.
Fossaria parva	–	–	18(I)	–	–	Roadside ditch, Clio, Genesee Co., Mich.
Fossaria modicella	36	–	18(I)	–	–	Parker's Mill, Washtenaw Co., Mich.
"	–	–	18(I, II)	–	–	Burnt Cabin Point, Huron Co., Mich.
"	36	–	18(I)	–	–	Comstock, Kalamazoo Co., Mich.
Fossaria rustica	38	38	19(I, II)	–	–	*Mill pond, near Dexter, Washtenaw Co., Mich.
"	–	–	19(I)	–	–	Willys Park, Toledo, Ohio
Radix natalensis	34	–	17(I)	–	–	Liberia, West Africa
'Lymnaea' tomentosa	32	–	16(I, II)	–	–	Australia
'Lymnaea' lessoni	–	–	16(I)	–	–	Papua

*The same locality as Burch (1960a, b).

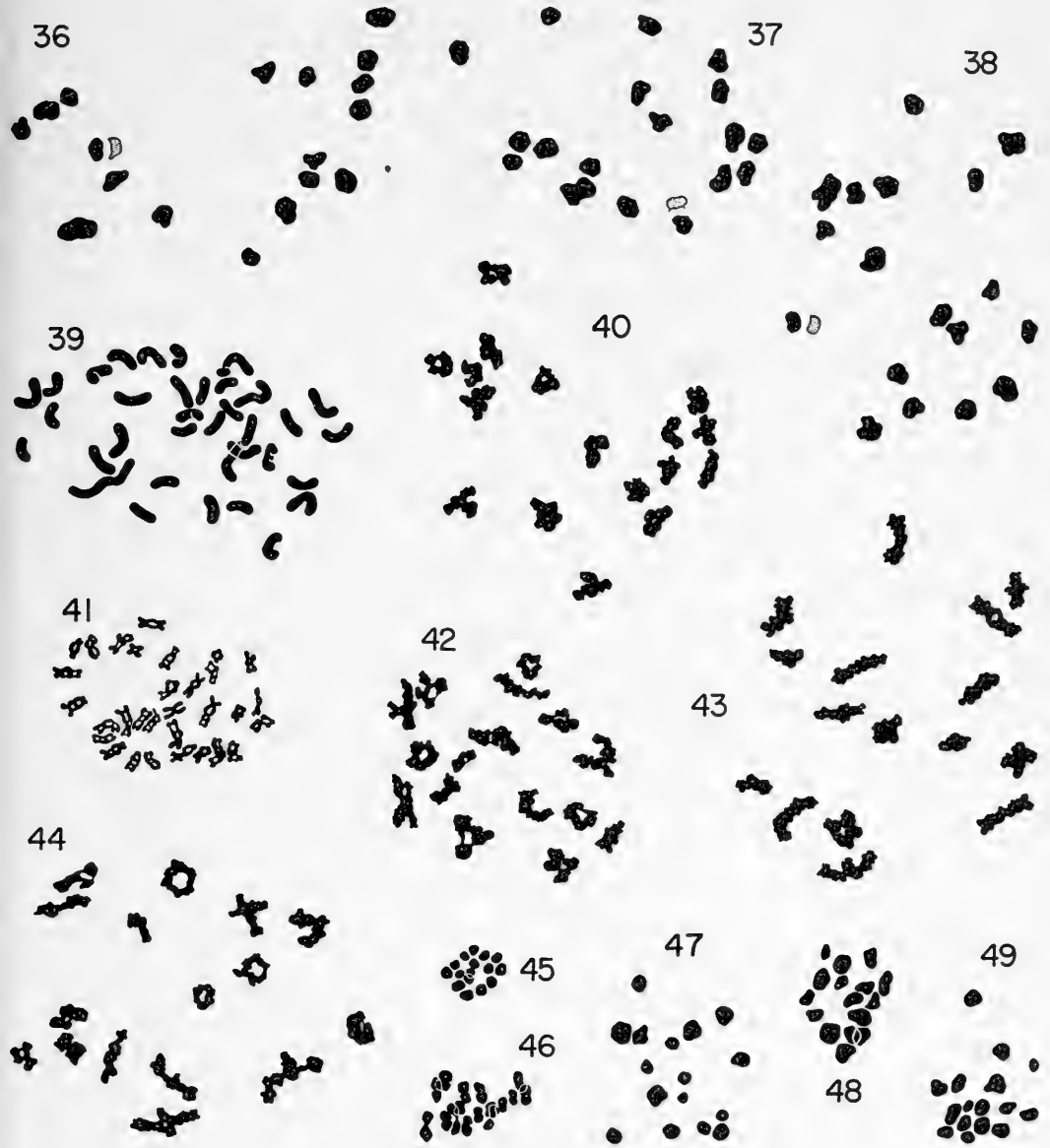
note: spg = spermatogonia. som = somatic mitosis. spe = spermatocyte. oc = oöcyte. pb = polar body.



FIGS. 1–17. Chromosomes in spermatogenesis. FIGS. 1, 2. *Stagnicola palustris wyomingensis* (Giggey Lake). FIGS. 3, 4. *S. p. wyomingensis* ("Lodge of the Pines"). FIGS. 5–7. *S. umbrosa*. FIG. 8. *S. exilis* Dancer & Trinkle Rds.). FIG. 9. *S. exilis* (Dancer & Jackson Rds.). FIGS. 10, 11. *S. catascopium* (Au Sable R.). FIGS. 12, 13. *S. catascopium* (Hammond Bay). FIGS. 14, 15. *S. emarginata serrata*. FIGS. 16, 17. *S. hinkleyi*. Figs. 5, 12 and 14 are of spermatogonial metaphase; Figs. 1, 4, 6–8, 10, 13, 15 and 16 are diakinesis chromosomes; Figs. 2 and 9 are 1st prometaphase chromosomes; Figs. 3 and 11 are of 1st metaphase; Fig. 17 is 2nd metaphase. Scale: 5 microns.



FIGS. 18-35. Chromosomes in spermatogenesis. FIG. 18. *Stagnicola idahoensis*. FIGS. 19, 20. *S.* cf. *bonnevillensis*. FIGS. 21, 22. *Pseudosuccinea columella*. FIG. 23. *Fossaria parva*. FIGS. 24, 25. *F. modicella* (Parker's mill). FIGS. 26-28. *F. modicella* (Burnt Cabin Point). FIGS. 29, 30. *F. modicella* (Comstock). FIGS. 31-35. *F. rustica* (Dexter). Figs. 18, 19, 21, 24, 29 and 31 of spermatogonial metaphase; Figs. 20, 23, 25, 26, 32 and 33 are diakinesis chromosomes. Figs. 22, 27, 30 and 34 are of 1st metaphase; Figs. 28 and 35 are of 2nd metaphase. Scale: 5 microns.



FIGS. 36-49. Chromosomes in spermatogenesis. FIGS. 36-38. *Fossaria rustica* (Toledo). FIGS. 39, 40. *Radix natalensis*. FIGS. 41-46. *"Lymnaea" tomentosa*. FIGS. 47-49. *"Lymnaea" lessoni*. Figs. 39 and 41 are of spermatogonial metaphase; Figs. 36-38, 40, 42-44 are diakinesis chromosomes; Figs. 47-49 are of 1st metaphase; Figs. 45 and 46 are of 2nd metaphase. Scale: 5 microns.

show the same appearance as those studied from Virginia (Burch, 1960b).

3. *Fossaria*

The chromosomes of 3 *Fossaria* species from 6 localities were observed. *Fossaria parva* (Fig. 23) and *F. modicella* (Figs. 24-30) have chromosome numbers of n=18 and 2n=36. But *F. rustica* has 19 haploid chromosomes and 38 chromosomes in gonial cells. Burch (1960b) reported the same chromosome number for this species from Michigan. The author confirmed it in the materials collected from the same locality (Figs. 31-35), and found in addition that this species from Toledo, Ohio also has 19 haploid chromosomes (Figs. 36-38). In diakinesis of *F. rustica*, one small bivalent was observed (Figs. 32, 33, 36-38). Generally this chromosome stained weakly.

4. *Radix*

Radix natalensis has 34 chromosomes in its spermatogonia (Fig. 39) and 17 chromosomes in meiotic cells (Fig. 40). This is the same number previously reported in *Radix*.

5. Others

This is the group which has 16 pairs of chromosomes. *"Lymnaea" tomentosa* from Australia and *"Lymnaea" lessoni* from Papua are included in this group. *"L." tomentosa* (Figs. 41-46) has 32 chromosomes in spermatogonial divisions and 16 chromosomes in the 1st and 2nd meiotic divisions. In *"L." lessoni* (Figs. 47-49), 16 haploid chromosomes were also observed. The caryotypes of these 2 species are similar to *"Lymnaea" ollula* (=*viridis*?) (Burch, et al., 1964; Inaba, 1965).

II. Chromosomes in oögenesis.

Chromosomes during oögenesis have been studied in 2 species of *Stagnicola*. Within half an hour after spawning, the extrusion of the polar body could be seen. In *S. palustris wyomingensis* 18 chromosomes were observed in the 1st oöcyte (Fig. 50) and the 1st polar body (Figs. 51, 52), as well as in the spermatocytes (Figs. 1-4). These chromosomes are all meta- or submetacentric in nature. In *S. exilis*, 18 chromosomes were also determined in the oöcyte (Fig. 53) and 1st polar body (Fig. 54). These chromosomes are also meta- or submetacentric in shape.

III. Chromosomes in somatic mitoses.

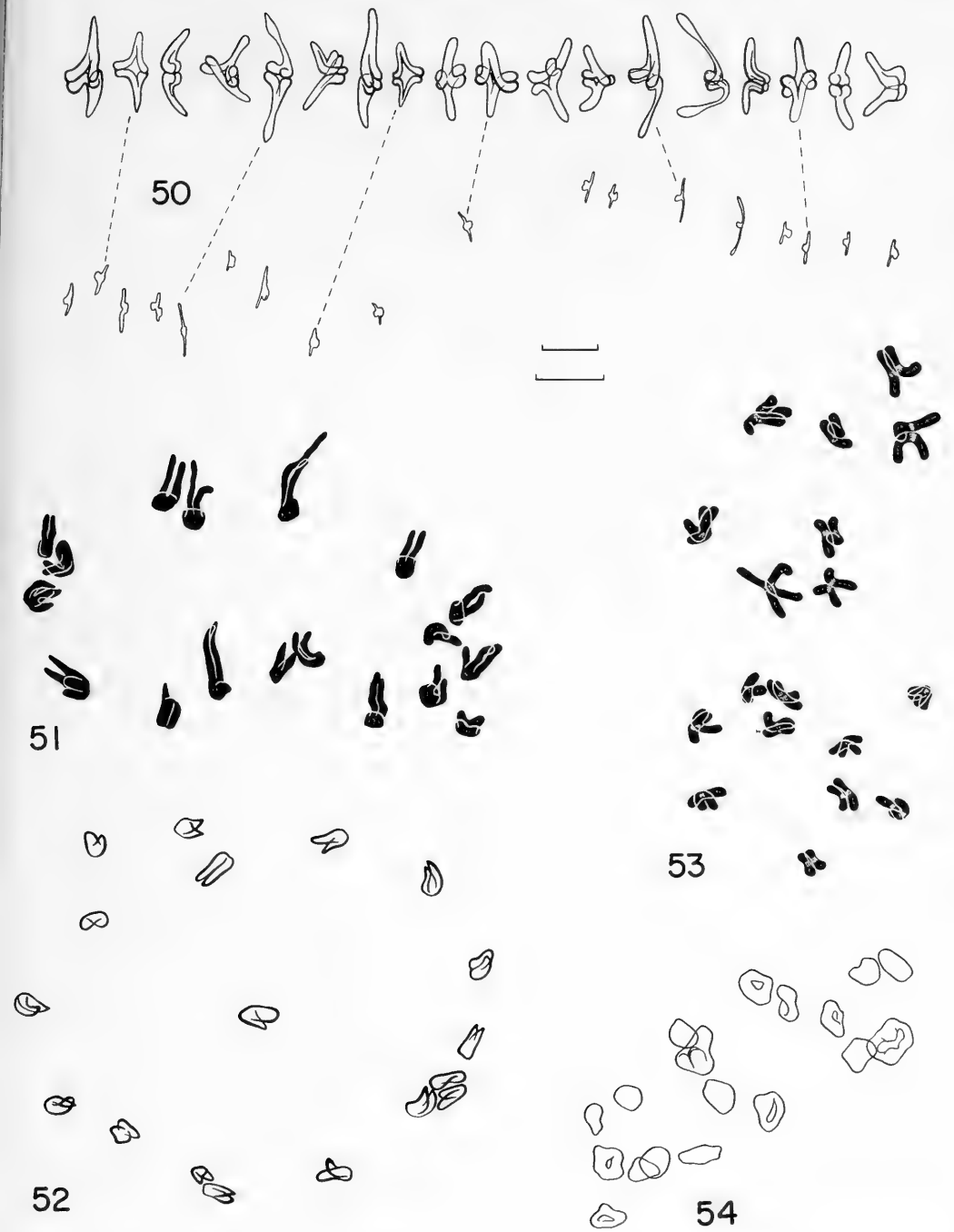
The mitotic chromosomes of 3 species of *Stagnicola*, *Bulimnea megasoma* and *Fossari rustica* have been studied during egg cleavage. Eighteen pairs of V- or J-shaped chromosomes were observed in *S. palustris wyomingensis* (Fig. 55), *S. exilis* (Figs. 56-59) and *S. catascopium* (Figs. 60-63). There is no significant observable difference among these 3 caryotypes. Two or 3 pairs are metacentric chromosomes, and the rest are submeta- or subtelocentric. However, it is difficult to consistently determine the exact nature of each chromosome, because the size and shape of chromosomes sometimes appear to be changed somewhat by the pressure of squashing (Figs. 56-57 and 60-61).

In *Bulimnea megasoma* (Fig. 64), 18 pairs of V- or J-shaped chromosomes were observed similar to those seen in *Stagnicola* species.

In *Fossaria rustica*, one more pair of chromosomes, i.e., 19 pairs, were recognized in the mitotic divisions of the embryos (Figs. 65-67).

DISCUSSION

In the Lymnaeidae, chromosome numbers have now been reported for 41 species belonging to 7 genera (Table 2). *Lymnaea*, *Stagnicola*, *Acella*, *Pseudosuccinea* and *Bulimnea* have the haploid chromosome number 18. The species of *Fossaria* have mostly the haploid



FIGS. 50-54. Chromosomes in oögenesis. FIGS. 50-52. *Stagnicola palustris wyomingensis* ("Lodge of the Pines"). FIG. 53. *S. exilis* (Dancer & Trinkle Rds.). FIG. 54. *S. exilis* (Dancer & Jackson Rds.). Figs. 50 and 53: 1st metaphase. Figs. 51, 52 and 54: 1st polar body chromosomes. In Fig. 50, the lower figure shows the whole nuclear plate (side view) in lower magnification. Lower scale: 5 microns. Upper scale (only for lower part of Fig. 50): 10 microns.

TABLE 2. Chromosome numbers reliably reported in Lymnaeidae

Species	Chromosome number				Source	Reference
	2n		n			
	spg	som	spc	oöc		
Lymnaea						
L. stagnalis	–	–	18	–	England	Burch, 1965
L. s. lacustris	36	–	18	–	Switzerland	Perrot, 1930
L. s. rhodani	36	–	18	–	Switzerland	Perrot, 1930, 1934
L. s. jugularis	36	–	18	–	Michigan, USA	Burch, 1960b
Stagnicola						
S. palustris	–	–	18	–	Switzerland;	Perrot & Perrot, 1938;
	–	–	18	–	Sweden	Burch, 1960b
S. p. elodes	36	–	18	–	Michigan, USA	Burch, 1960b
S. p. desidiosa	36	–	18	–	Michigan, USA	Burch, 1960b
S. p. wyomingensis	–	36	18	18	Colorado, USA	Inaba (this paper)
S. umbrosa	36	–	18	–	Michigan, USA	Burch, 1960b; Inaba (this paper)
S. exilis	–	36	18	18	Michigan, USA	Burch, 1960b; Inaba (this paper)
S. catascopium	–	36	18	–	Michigan, USA	Burch, 1960b; Inaba (this paper)
S. reflexa	–	–	18	–	Ohio, USA	Burch, 1960b
S. lanceata	–	–	–	18	Minnesota, USA	Burch, 1960b
S. emarginata serrata	36	–	18	–	Michigan, USA	Burch, 1960a,b; Inaba (this paper)
S. hinkleyi	–	–	18	–	Wyoming, USA	Inaba (this paper)
S. idahoensis	36	–	–	–	Idaho, USA	Inaba (this paper)
S. cf. *bonnevillensis*	36	–	18	–	Wyoming, USA	Inaba (this paper)
S. (Hinkleyia) caperata	–	–	18	–	Ohio, USA	Burch, 1960b
S. (H.) montanensis	36	–	18	–	Idaho, USA	Burch, 1963
Acella						
A. haldemani	36	–	18	–	Michigan, USA	Burch, 1960b
Pseudosuccinea						
P. columella	36	–	18	–	Va., Mich., USA	Burch, 1960b; Inaba (this paper)

spg = spermatogonia; som = somatic mitosis; spc = spermatocyte; oöc = oöcyte.

Table 2 (cont.)

Species	Chromosome number				Source	Reference
	2n		n			
	spg	som	spc	oöc		
Bulimnea						
B. megasoma	36	36	18	–	Michigan, USA	Burch, 1960a,b; Inaba (this paper)
Fossaria						
F. parva	–	–	18	–	Michigan, USA	Burch, 1960a,b; Inaba (this paper)
F. modicella	36	–	18	–	Ohio, Mich., USA	Burch, 1960b; Inaba (this paper)
F. truncatula	36	–	18	–	Japan	Burch, et al., 1964
F. sp. (= *truncatula*)	–	–	18	–	Japan	Burch, 1965
F. rustica	38	38	19	–	Mich., Ohio, USA	Burch, 1960b; Inaba (this paper)
Radix						
R. auricularia	–	–	17	–	Switzerland	Perrot & Perrot, 1938
R. a. swinhoei	–	–	17	–	Formosa	Burch & Natarajan, 1965
R. a. japonica	34	–	17	–	Japan	Burch, et al., 1964; Inaba, 1965
R. ovata	–	–	17	–	Switzerland	Perrot & Perrot, 1938
R. peregra	–	–	17	–	Switzerland	Perrot & Perrot, 1938
	34	–	17	–	Turkey	Burch, 1960b
R. onychia	34	–	17	–	Japan	Burch, et al., 1964
R. luteola	34	–	17	–	India	Natarajan, 1960
R. hovarum	–	–	17	–	Madagascar	Burch, 1965
R. sp.	–	–	17	–	Italy	Burch, 1965
R. natalensis	34	–	17	–	Liberia	Inaba (this paper)
"*R. limosa*"	36	–	18	–	Europe	La Calvez & Certain, 1950
"*Lymnaea*"						
"*L.*" ("*Radix*") *ollula* (= *viridis?*)	32	–	16	–	Japan	Burch, et al., 1964; Inaba, 1965
"*L.*" *tomentosa*	32	–	16	–	Australia	Inaba (this paper)
"*L.*" *lessoni*	–	–	16	–	Papua	Inaba (this paper)

number 18, but *F. rustica* has 19. All species of *Radix* have 17 pairs of chromosomes. *"Lymnaea" tomentosa* and *"L." lessoni* have the same chromosome number, n=16 as *"L." ollula* (=*viridis*?).

The use of spermatogonial or spermatocyte chromosomes as taxonomic characters at the level of the lower taxonomic categories is very difficult because these chromosomes are small and do not show remarkably diverse characters. The majority or all of the spermatogonial metaphases of all the species examined had elongate chromosomes that were medianly or submedianly constricted. However, the chromosomes of the mitotic divisions in early development of the embryos are very useful for caryotype analysis because their size and shape are larger and longer than the spermatogonial chromosomes. Since the size and shape of the chromosomes are changed rather easily by the pressure of squashing (see Figs. 56-57 and 60-61), it is very difficult at present to get constant figures of chromosomes. But this variability seems to be controlled by the time of fixation or staining. At any rate, such studies show considerable promise as an aid toward clarifying systematics in the lower taxonomic categories.

In the following paragraphs, I wish to discuss some taxonomic points based on cytological studies in each chromosome number group, and possible phylogentic relationships within the Lymnaeidae.

I. Cytotaxonomic considerations in Lymnaeidae.

1. *Stagnicola* and others, 18(n) groups (except *Fossaria*).

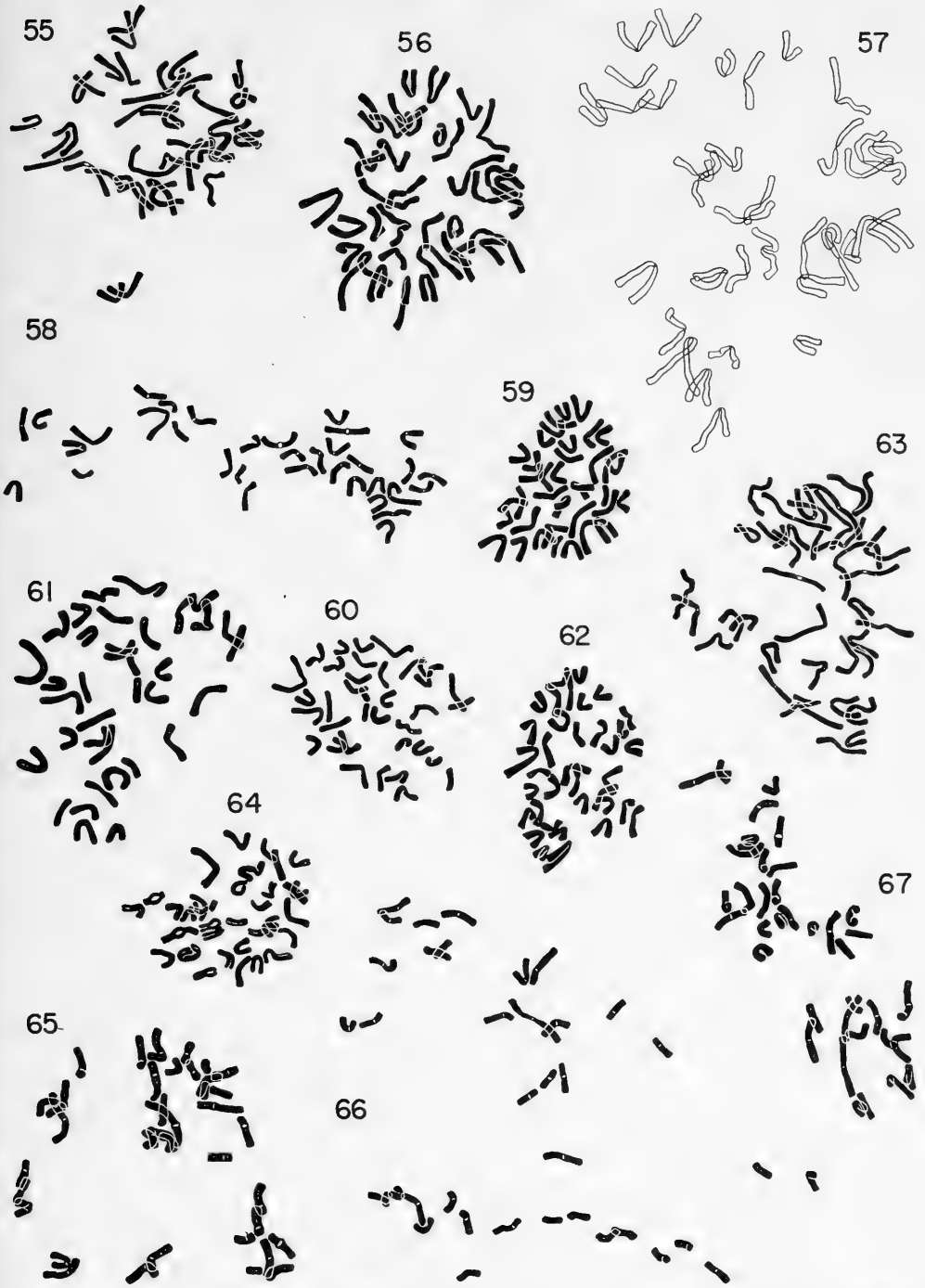
Stagnicola is the most extensively studied generic group. The chromosomes of 15 species and subspecies have been observed. All of them have 18 pairs of chromosomes. *Lymnaea*, *Acella*, *Pseudosuccinea* and *Bulimnea* also have 18 pairs of chromosomes. No distinct morphological difference or remarkable characteristics have been found between the caryotypes of these genera. It is difficult to analyze spermatogonial caryotypes, but from observations on somatic chromosomes, it may be possible to use the latter caryotype for comparisons at the species level. However, in order to critically analyze such caryotypes, more detailed and numerous observations are desirable. At present, in comparing the somatic caryotypes of 3 *Stagnicola* species, the author wishes to point out only the following few features: (1) Generally, most of the chromosomes are meta- or submetacentric in nature, but in *S. palustris wyomingensis*, the 11th chromosome is more or less subtelocentric; (2) The chromosomes decrease gradually in length from the 2nd down to the last (18th), but the 1st chromosome is noticeably longer than the rest. The 2nd chromosome is 83% of the length of the 1st one in *S. palustris* wyomingensis, but only 80% in *S. exilis* and 72% in *S. catascopium* (Figs. 68-70).

Stagnicola species have bicuspid lateral radular teeth (Figs. 71-74). The genus *Lymnaea* also has bicuspid laterals, but the 1st lateral occasionally has 3 cusps. Other genera, e.g., *Acella*, *Pseudosuccinea* and *Bulmnea*, have tricuspid lateral teeth. Hubendick (1951) considers that there is nothing with definite taxonomic importance among these generic names. However, I find it convenient to classify the n=18 group into the above genera on characters of the shells, and morphology of the radulae and genitalia.

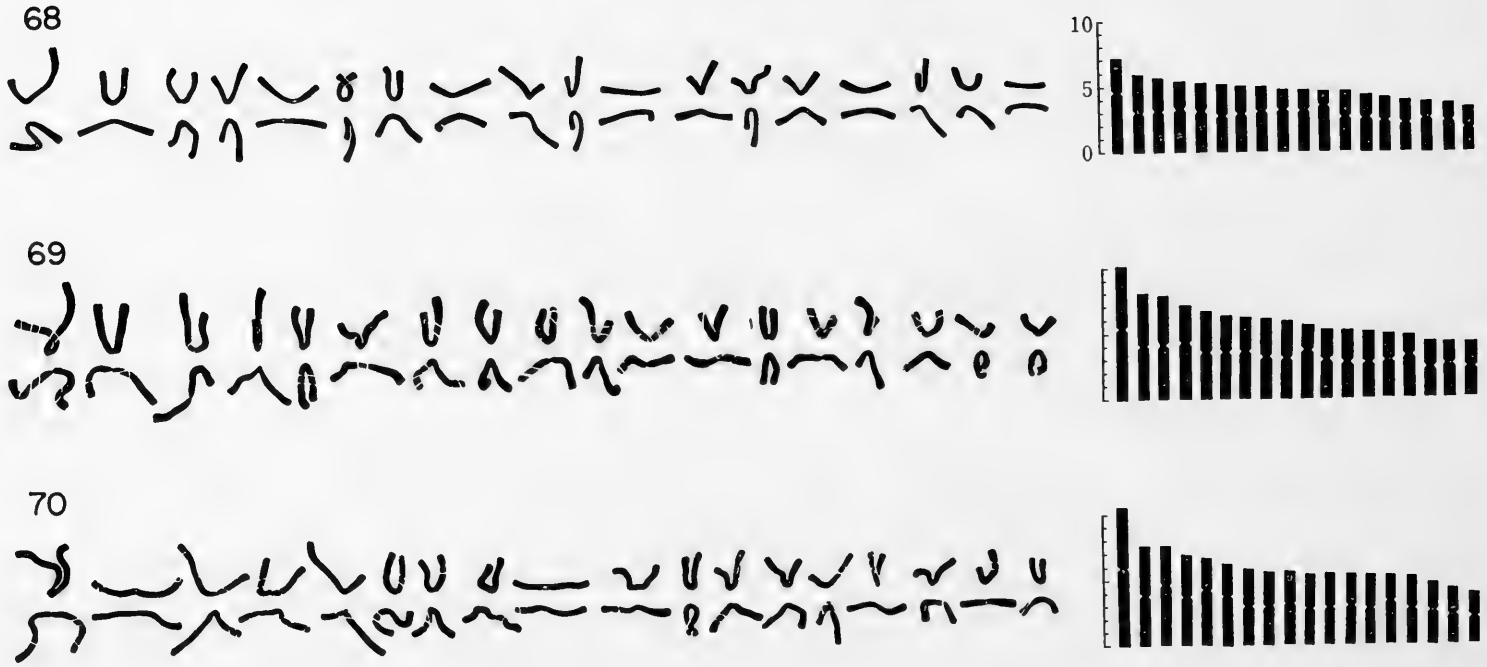
2. *Fossaria*[3], 18(n), 19(n) group.

The chromosomes of 5 species have been observed. These species have 18(n) chromosomes as in the preceding

[3]The generic name *Fossaria* Westerlund 1885 is used here instead of *Galba* Schranck 1803, according to the opinion of Baker (1928).



FIGS. 55–67. Chromosomes in somatic mitosis. FIG. 55. *Stagnicola palustris wyomingensis* (Giggey Lake). FIG. 56, 57. *S. exilis* (Dancer & Trinkle Rds.). FIG. 57. The same chromosomes as in Fig. 56, but pressed out more. FIGS. 58, 59. *S. exilis* (Dancer & Jackson Rds.). FIGS. 60–63. *S. catascopium* (Au Sable R.). FIG. 61. The same chromosomes as in Fig. 60, but pressed out more. FIG. 64. *Bulimnea megasoma*. FIGS. 65–67. *Fossaria rustica* (Dexter).



FIGS. 68–70. Serial arrangement and schematic representation of the somatic metaphase caryotypes of 3 *Stagnicola* species. FIG. 68. *Stagnicola palustris wyomingensis* (chromosomes shown in Fig. 55). FIG. 69. *S. exilis* (chromosomes shown in Fig. 56). FIG. 70. *S. catascopium* (chromosomes shown in Fig. 63). Scale: 10 microns.

group, with only one known exception (*Fossaria rustica*, n=19). Morphological differences in the chromosomes could not be found between *Fossaria* (except *F. rustica*) and the other 18(n) group. These small lymnaeid snails have tricuspid lateral teeth, without exception (Figs. 78-81).

Burch (1960b) observed that *Fossaria modicella rustica* has the haploid chromosome number 19 and considered it to have gained 1 bivalent in addition to the 18 of an original species, *F. modicella*. My studies on this species collected from the same locality confirm his observations, and in addition, I found that this same species from Ohio also has 19(n) chromosomes. The additional 19th chromosome was small and rather weakly stained in the 1st spermatocyte diakinesis. These cytological features indicate that *F. rustica* is not a variety of *F. modicella*, but a new species which originated (probably) from *F. modicella* by the addition of 1 bivalent, i.e., by natural aneuploidy (hyperploidy). As such, *F. rustica* seems to be a species that has appeared rather recently. It originally had been described by Lea (1841) as *Lymnea rustica;* Baker (1928) considered it as a variety of *F. modicella*. Body features, genitalia, jaw and radula seem to be similar in all respects to those of *F. modicella*. The ecological habitat is also similar to *F. modicella*. Hubendick (1951) stated that *"L." parva* Lea, *"L." humilis modicella* Say, *"L." humilis rustica* Lea, and similar fossarid species in Baker's (1911) monograph must probably be linked to *"Lymnaea" humilis* Say, according to characteristics of the shell, radula and genitalia. But he mentioned in his preceding part that the first laterals of *"L." humilis* were bicuspid (in his figure, the 2nd lateral is also bicuspid). Therefore, it would seem that his material, at least that from California, was probably small specimens of some *Stagnicola* species.

The caryotypes of many *Fossaria* species have not yet been studied. Accordingly, other species possessing 19(n) chromosomes yet may be found. At any rate it is a very interesting feature that a snail possessing one more bivalent has been found in the genus *Fossaria*. This number is the highest among the Lymnaeidae.

3. *Radix*, 17(n) group.

The chromosomes of 11 species and subspecies of *Radix* have been studied, all of which have 17 pairs of chromosomes. In the Lymnaeidae, this number is restricted to this genus[4]. Previously, Burch, et al. (1964), Burch (1965) and Inaba (1965) considered *"Lymnaea" ollula* (=*viridis*?) to belong to *Radix*, because it seemed cytologically closer to this genus than to *Fossaria*. But I now consider it to belong to another generic group, which is characterized by only 16 pairs of chromosomes (see below).

Lymnaea natalensis has 17 pairs of chromosomes and is also a member of the genus *Radix*. Fifty or more species and forms of Lymnaeidae have been described from Africa. However, Germain (1919) has reduced the number to 6,

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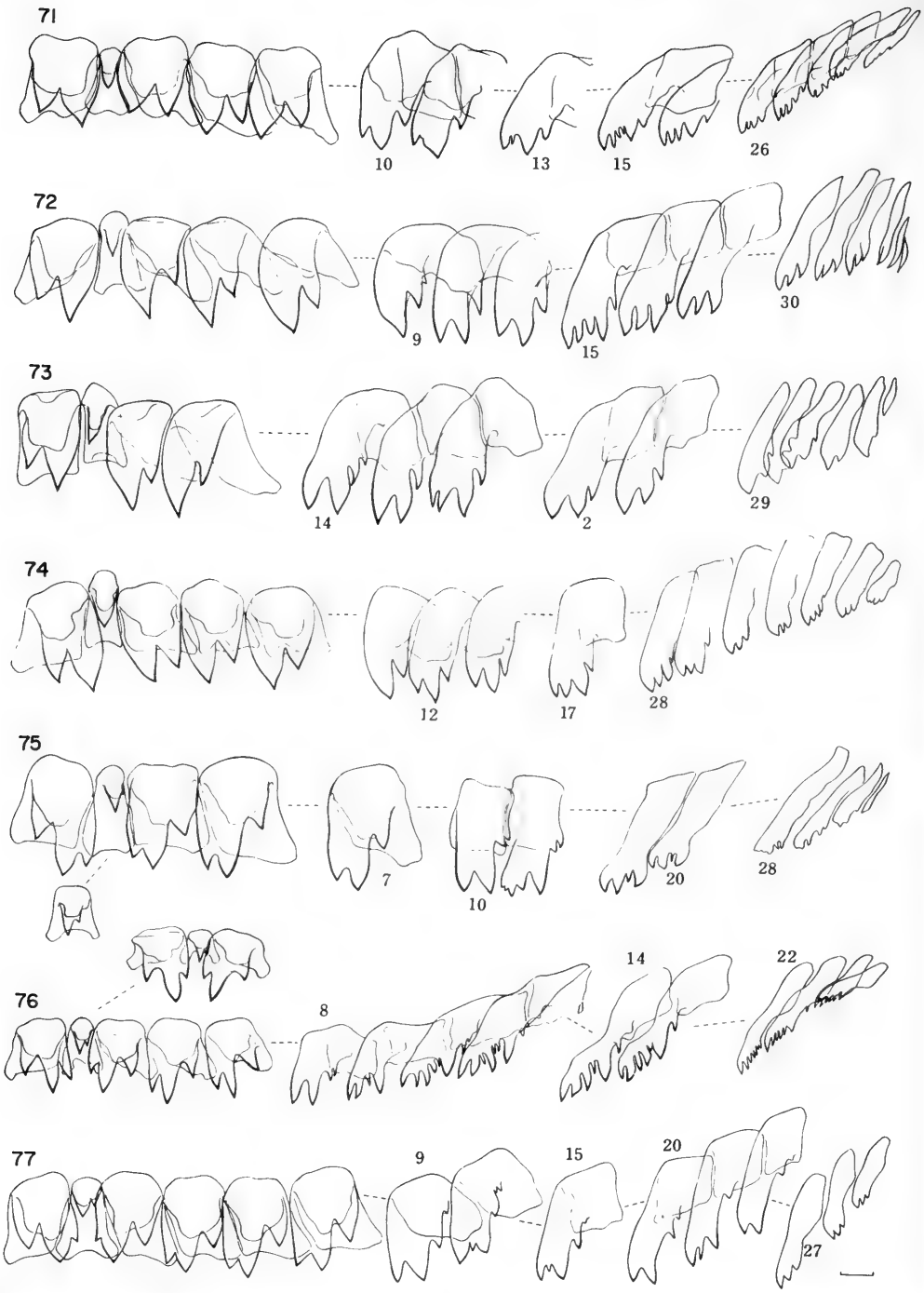
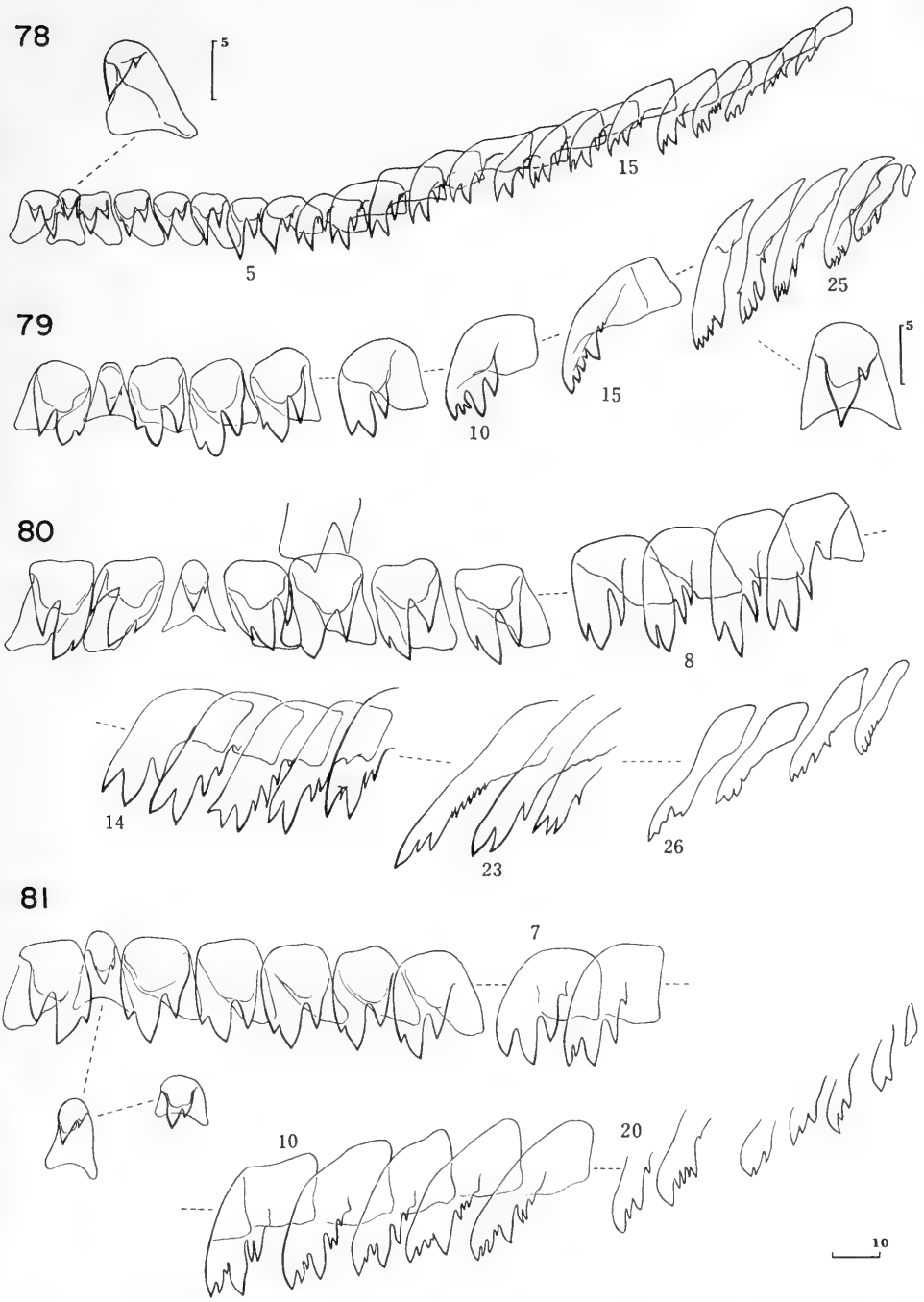
4 *"Radix limosa"* in Europe has been reported to have 18 pairs of chromosomes (Le Calvez & Certain, 1950), but Burch (1965) explained that it is uncertain whether Le Calvez & Certain's *"limosa"* was *R. balthica* (L.) or some other species of *Radix*, because the true *Helix limosa* Linnaeus, 1758 is not a lymnaeid. Hubendick (1951) also writes that probably it was not a *Lymnaea* species originally, but in the literature it is used for *L. peregra* Müller or *peregra auricularia* L. Further, he says *Helix balthica* Linnaeus 1758 is also probably not *Lymnaea*, but used for *P. peregra* by mistake in the literature. At any rate, Le Calvez and Certain's species, whatever it is, should be re-examined.



FIGS. 71–77. Radulae of Lymnaeidae. FIG. 71. *Stagnicola palustris wyomingensis* (Giggey Lake). FIG. 72. *S. catascopium* (Au Sable R.). FIG. 73. *S. emarginata serrata*. FIG. 74. *S. hinkleyi*. FIG. 75. *Radix natalensis*. FIG. 76. *"Lymnaea" tomentosa*. FIG. 77. *"Lymnaea" lessoni*. Scale: 10 microns.



FIGS. 78-81. Radulae of Lymnaeidae. FIG. 78. *Fossaria parva*. FIG. 79. *F. modicella* (Burnt Cabin Point). FIG. 80. *F. rustica* (Dexter). FIG. 81. *F. rustica* (Toledo). Scale: 10 microns, except the figures of 2 central teeth in Figs. 78, 79.

and Hubendick (1951) reduced the number of species still further, to 1 species, *L. natalensis* Krauss 1848. The latter is the oldest lymnaeid name described from Africa, except *L. aegyptica* Bourguignat 1883 (nom. nud.). However it is problematical whether all of those nominal species or forms are only 1 species or perhaps more. It would be desirable to study their chromosomes. The chromosome number of *R. natalensis* is n=17, as in other *Radix* species previously studied. Hubendick (1951) says that *L. natalensis* ought to be linked with the superspecies *L. auricularia*, an opinion which coincides with my cytological results. *Radix hovarum* (Tristram 1863) from Madagascar (n= 17, Burch 1965) is probably also a form of *R. natalensis*, or a species closely related to it.

4. The 16(n) group.

At present, 3 lymnaeid species are known to have only 16 pairs of chromosomes. These are *"Lymnaea" ollula* (=*viridis?*), *"L." lessoni* and *"L." tomentosa*.

"Lymnaea" tomentosa was originally described as a *Succinea* by Pfeiffer (1855). About 10 species and subspecies in its lymnaeid group have been described from New Zealand, Tasmania and Southern Australia. Hubendick (1951) considered that if further investigations should prove *L. tomentosa* from New Zealand to be specifically distinguishable from that of Australia and Tasmania, the latter ought to be called *L. aruntalis* for which Cotton (1942) established the generic group name *Austropeplea* (type: *L. aruntalis* Cotton & Godfrey 1938, new name for *L. papyracea* Tate 1880 from South Australia).

From New Guinea and Papua to New Zealand, except the southwestern parts of Australia, *"L." lessoni* (Deshayes 1831) occurs. About 20 forms have been reported in this species group.

Between the geographical distributions of the above 2 species groups and *"Lymnaea" viridis*, at least 3 species are distributed: (1) *"L." cumingiana* (Pfeiffer 1853) (Luzon, Philippines), (2) *"L." buruana* (Haas 1913) (Lake Wakolo, Central Buru, Moluccas), and (3) *"L." brevispira* (Martens 1897) (Lake Manindjan, Sumatra). However these species have not yet been studied cytologically.

Burch (1965) pointed out that *"Lymnaea" ollula* (=*viridis?*) perhaps should be placed in the genus *Radix* instead of the genus *Fossaria*, because its chromosome number (n=16) is closer to *Radix* (n=17) than to *Fossaria* (n=18, 19). Further, Burch (1967) stated regarding this snail (as a suggestion from Dr. Yoshio Kondo) that "perhaps *ollula* (=*viridis?*) is an archaic form that entered the Pacific with the Orthurethra during the Late Paleozoic to Early Mesozoic, and gave rise to such species as *Lymnaea volutata* of Hawaii, *L. brevispira* of Sumatra, *L. buruane* of Indonesia, *L. cumingiana* of the Philippines and *L. lessoni* of Australia, New Guinea and New Zealand." I support this opinion.

But as regards the generic name, the author does not accept the use of *Bakerilymnaea* for *"Lymnaea" ollula* (=*viridis?*), because *Bakerilymnaea* Weyrauch 1964 was established as a new name for the preoccupied subgenus *Nasonia* F. C. Baker 1928 (non *Nasonia* Ashmead 1904; Hymenoptera, Insecta) in the genus *Stagnicola* Leach 1840, and the type species is *Lymnaea cubensis* Pfeiffer 1839 from Cuba (Weyrauch, 1964). The latter has bicuspid lateral teeth similar to *Stagnicola*, and *Stagnicola* has 18(n) chromosomes as mentioned above, while *"L." ollula* (=*viridis?*) has tricuspid laterals and 16(n) chromosomes. Therefore *Bakerilymnaea* should be used only for *L. cubensis* and its allied species.

The longitudinal geographical range of the group with 16(n) chromosomes extends from the eastern part of Asia to Australia. It is a very interesting problem as to whether or not this unique distribution occurred for geological reasons. Further cytological studies on

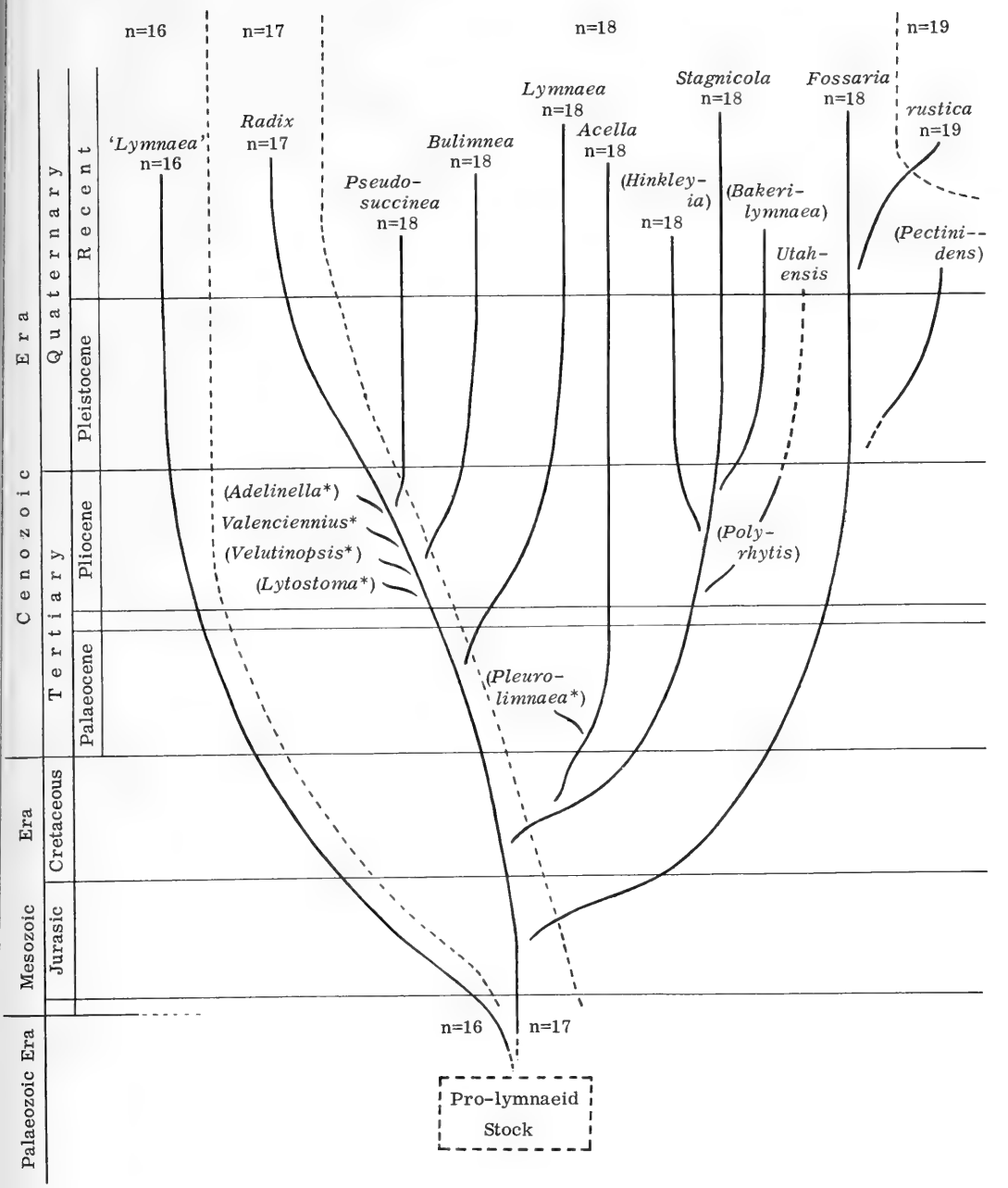


FIG. 82. Hypothetical phylogenetic diagram of the lymnaeid genera and subgenera based partly on haploid chromosome numbers, with consideration of fossil groups and geological data. * marks fossil groups not found in the Recent. Paleontological information from Zilch (1959-60, Gastropoda, Teil 2, Euthyneura, *In:* Schindewolf, Handbuch der Paläozoologie, v. 6, Borntraeger, Berlin, xii + 834 p (p 91-102)).

these geographical groups are desirable.

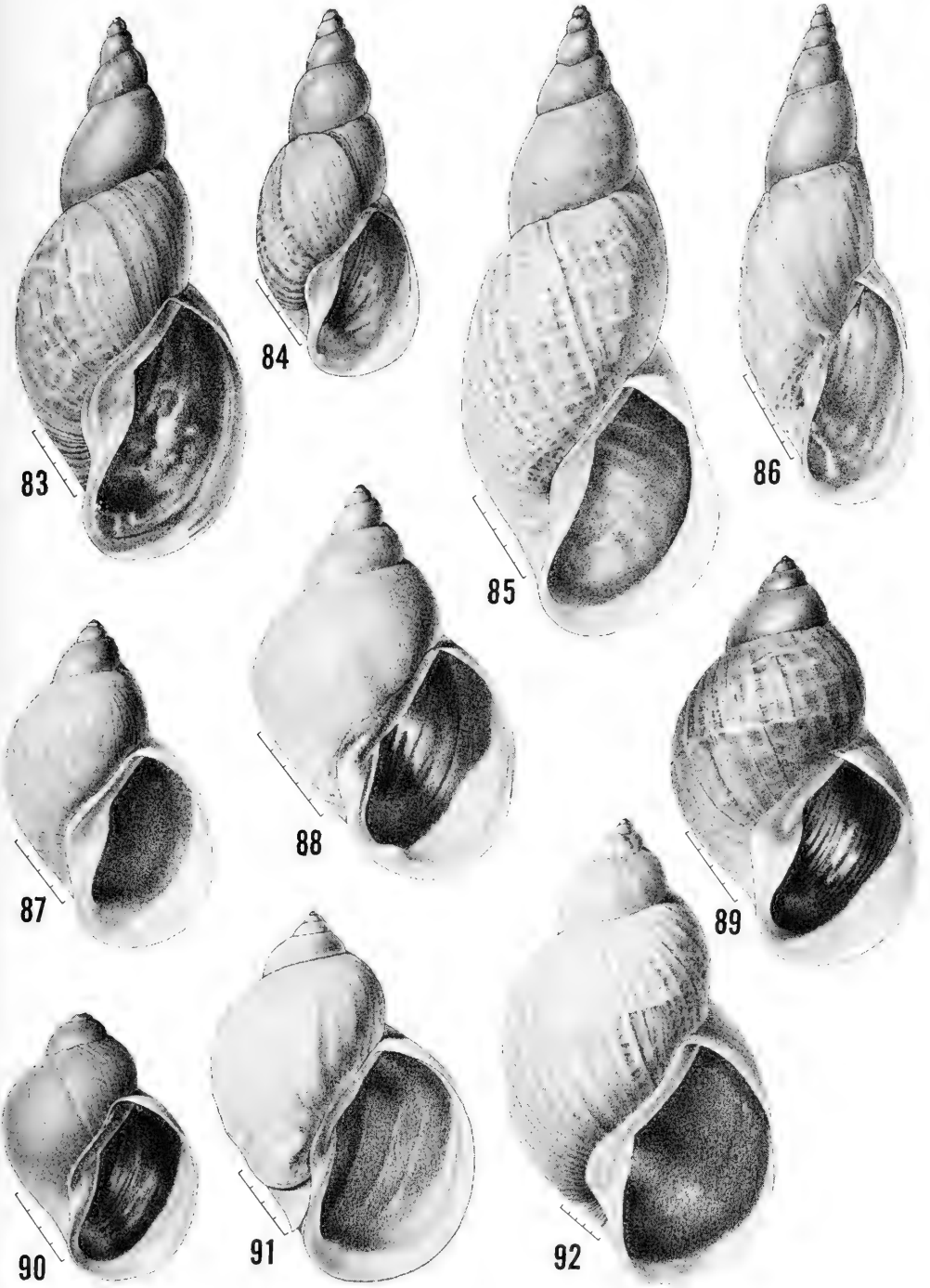
A separate generic name may be needed for the 16(n) group. If *Lymnaea aruntaris* of Australia is congeneric with *L. tomentosa* of New Zealand, then *Austropeplea* Cotton 1942 (Trans. Roy. Soc. S. Austr., 66: 80) would be the valid generic name for this group. *Amphipeplea* Nilsson 1822 (=*Myxas* Sowerby 1822; type: *Buccinum glutinosa*, Müller 1774, Europe) has been used in the past for many forms of *L. lessoni* and *L. tomentosa*, but these species differ in many characteristics from the European *L. glutinosa*. *Peplimnea*, *Simlimnea* and *Glacilimnea* of Iredale (1943) would seem to be synonyms of *Austropeplea*.

II. Phylogenetic considerations in Lymnaeidae.

Hubendick (1951) revised the Lymnaidae of the world primarily on the shell and on a few characteristics of the male reproductive tract. As a result, he reduced nearly 1,800 specific names to approximately 40, and included all of them in 2 genera: *Lymnaea* and *Lanx*. In the Lymnaeidae, the anatomical similarity among species and the variations within one species create a number of unusual taxonomic problems which seem to obscure systematic relationships. Hubendick (1951, p 110) concluded from his morphological studies that " . . . no clear evolutionary lines [have] appeared and none have, in any case, been proved. It is impossible to construct a plylogenetic diagram even of the main relations within Lymnaeidae." However, I consider that chromosome numbers and the nature of mitotic figures can be useful in clarifying lymnaeid systematic relationships, and the occurrence of 3 different chromosomal groups verifies this.

Burch (1965; 1967, p 122) considered that "the original pro-limnaeid stock had less than 18 chromosomes, and that this stock gave rise to *Radix* which gained wide geographic distribution retaining 17 pairs of chromosomes, but one or more times certain populations gained a bivalent. From an early pro-*Radix* stock (or perhaps from *Radix* itself) on one or more occasions there was a genetic separation of populations which gained an extra bivalent during or after the separation, resulting in one or more divisions of the family being characterized by 18 pairs of chromosomes." I agree with this opinion. Such speculation seems to be confirmed by the extra small bivalent in *Fossaria rustica* (Figs. 32, 33, 36-38). It seems likely that some *Fossaria* population (perhaps a population of the species *L. modicella*) gained a bivalent and subsequently a new reproductively isolated species (*F. rustica*) resulted. Burch (1965) presented a diagram of possible relationships of various taxa of the Lymnaeidae based mainly on haploid chromosome numbers. But since then, additional cytological data have become available. Therefore, I am presenting a revised phylogenetic diagram (Fig. 82) based on haploid chromosome numbers in conjunction with paleontological information.

The hypothetical pro-lymnaeid ancestor probably appeared in the Paleozoic era. The 16(n) group seems to be an archaic form, perhaps appearing in the late Paleozoic or early Mesozoic eras. The first to diverge from this stock was *Radix* (n=17). In the Jurassic era *Fossaria* (n=18) appeared. *Stagnicola* (n=18) probably originated from *Radix* stock in the Cretaceous era. Soon after this divergence, *Acella* (n=18) branched from the *Stagnicola* stem. In the Paleocene of the Cenozoic era, *Lymnaea* (n=18) branched from the *Radix* stem. *Pleurolimnaea* (fossil) branched in this time from the *Acella* stem, and many fossil groups of *Radix*, and *Polyrhytis* from *Stagnicola*, appeared in the Pliocene. From the middle to late Pliocene, *Hinkleyia* and *Bakerilymnaea* branched from the *Stagnicola* stem. At almost the same time, *Bulimnea* and *Pseudosuccinea* appeared, probably from the *Radix* stem. Recently a 19(n) species originated within the *Fossaria* group. The origin of



FIGS. 83–92. Shells of snails used in this study. FIG. 83. *Stagnicola palustris wyomingensis* (Giggey Lake). FIG. 84. *S. p. wyomingensis* ("Lodge of the Pines"). FIG. 85. *S. umbrosa*. FIG. 86. *S. exilis* (Dancer and Trinkle Rds.). FIG. 87. *S. catascopium* (Au Sable R.). FIG. 88. *S.* cf. *bonnevillensis*. FIG. 89. *S. hinkleyi*. FIG. 90. *S. idahoensis*. FIG. 91. *S. emarginata serrata*. FIG. 92. *Bulimnea megasoma*. Measurement lines in mm.

Pectinidens and its relationship to *Fossaria* is obscure.

The haploid number of the pro-lymnaeid stock was probably 16. However, if it had 17(n) chromosomes, then one bivalent was lost in the evolution of the 16(n) group. Further cytological studies on the Lymnaeidae should shed considerable light on this subject.

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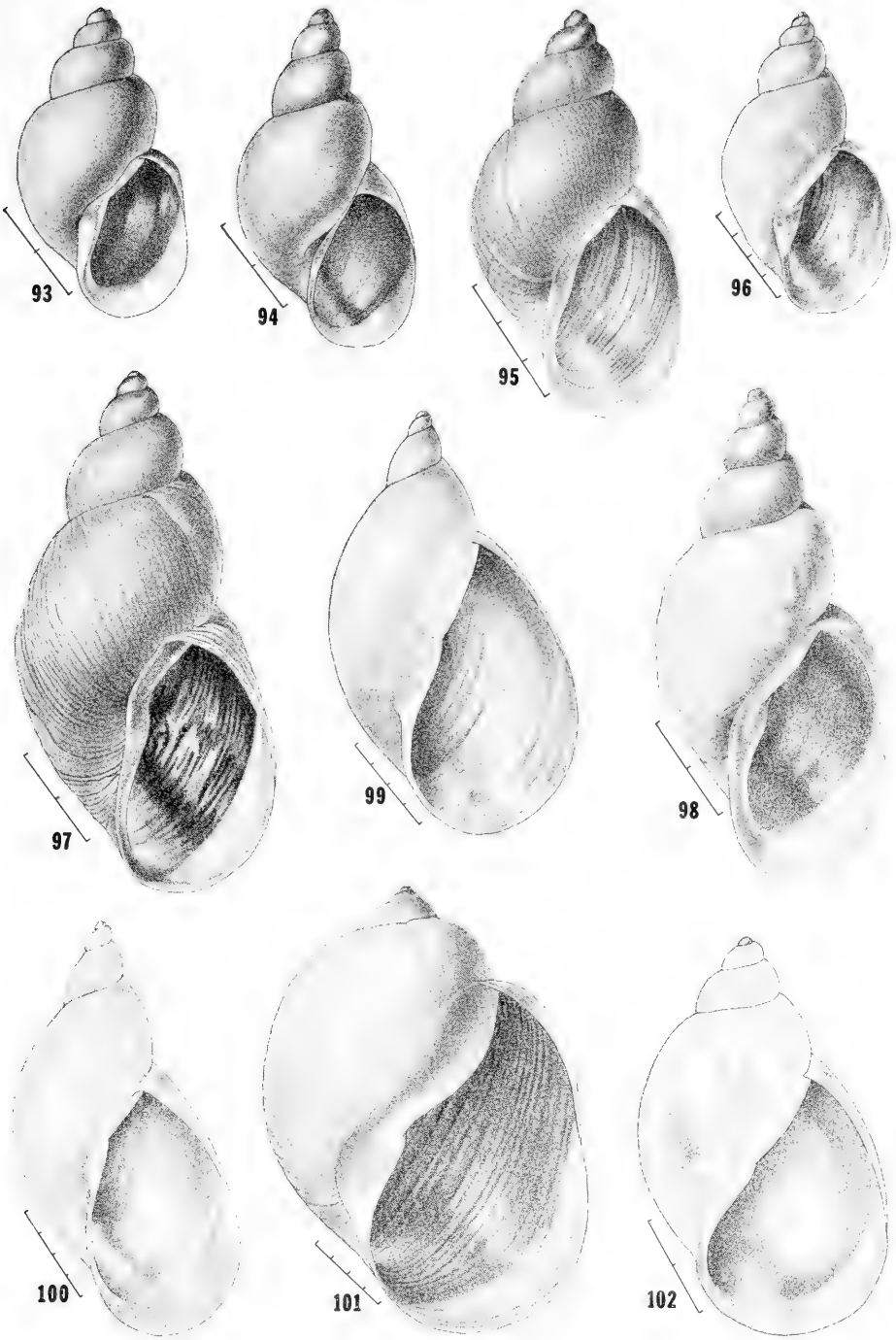

FIGS. 93-102. Shells of snails used in this study. FIG. 93. *Fossaria parva*. FIG. 94. *F. modicella* (Parker's mill). FIG. 95. *F. rustica* (Toledo). FIG. 96. *F. modicella* (Comstock). FIG. 97. *F. rustica* (Dexter). FIG. 98. *F. modicella* (Burnt Cabin Point). FIG. 99. *Radix natalensis*. FIG. 100. *Pseudosuccinea columella*. FIG. 101. *"Lymnaea" lessoni*. FIG. 102. *"Lymnaea" tomentosa*. Measurement lines in mm.

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RÉSUMÉ

ETUDES CYTOTAXONOMIQUES SUR DES LYMNÉES

A. Inaba

Les chromosomes de 16 espèces de Lymnaeidae originaires de 22 localités ont été observés durant la spermatogénèse ainsi que durant l'ovogénèse et les divisions mitotiques. Les chromosomes de 7 espèces et sous-espèces sont décrits pour la première fois dans cet article. Pour les autres espèces, des apports nouveaux par rapport aux études précédentes, concernent les chromosomes dans les spermatogonies, les ovocytes, les globules polaires et les mitoses somatiques. Les nombres de chromosomes déterminés sont comparés avec ceux qui ont été précédemment décrits. Chez les Lymnaeidae, on connait actuellement le nombre de chromosomes de 41 espèces et sous-espèces appartenant à 7 genres. En se basant sur des faits cytologiques, divers points de taxonomie sont discutés dans chaque groupe différant par le nombre de chromosomes.

Lymnaea (4 sous-espèces), *Stagnicola* (15 espèces et sous-espèces), *Acella* (1 espèce), *Pseudosuccinea* (1 espèce) et *Bulimnea* (1 espèce) ont tous 18 comme nombre haploïde. On n'a trouvé ni différence morphologique évidente, ni caractéristiques remarquables entre et parmi ces genres. Trois espèces de *Fossaria* ont le nombre haploïde 18, mais *F. rustica* a 19. Ceci suggère que cette dernière mérite le rang d'espèce, bien que beaucoup d'auteurs la considéraient comme une forme ou sous-espèce de *F. modicella*. La paire de chromosomes additionnelle est petite et souvent assez faiblement colorable pendant la diacinèse du spermatocyte.

Radix (11 espèces et sous-espèces) a comme nombre haploïde 17. *"Lymnaea" natalensis* du Libéria a aussi n=17, ce qui suggère que c'est une espèce du genre *Radix*. L'anatomie confirme cette opinion. Trois espèces de Lymnées ont seulement 16 (n) chromosomes: *"L." ollula* (=*viridis*?), *"L." tomensosa* et *"L." lessoni*. Elles ont été incluses dans différents groups génériques, surtout d'après leurs caractères conchyliologiques. Cependant, un nouveau nom de groupe doit être crée pour ces espèces à n=16.

Les chromosomes somatiques ont été observés chez 5 espèces: (*Stagnicola palustris wyomingensis*, *S. exilis*, *S. catascopium*, *Bulimnea megasoma* et *Fossaria rustica*). En général, tous les chromosomes dans les mitoses somatique de jeunes embryons sont méta- ou submétacentriques. On pourrait très bien utiliser ces chromosomes pour des analyses caryotypiques, mais des observations plus détaillées seront nécessaires avant de pouvoir esquisser des conclusions valables à partir d'études comparées.

Enfin, l'auteur expose des considérations sur la phylogénie des Lymnaeidae, basées sur des données cytotaxonomiques, cytologiques et paléontologiques.

RESUMEN

ESTUDIOS CITOTAXONOMICOS EN LIMNEIDOS

A. Inaba

Se observaron los cromosomas en 16 especies de limneidos, durante sus espermatogenesis, ovogenesis y divisiones mitósicas. Se informa por primera vez acerca de los cromosomas de 7 especies y subespecies, y para otras se agrega nuestro conocimiento a los ya previamente informados de la espermatogenesis, oocitos, cuerpos polares y mitosis somática. Se conoce hasta ahora el número cromosomático de 41

especies de Lymnaeidae (con subespecies), pertenecientes a 7 géneros. En base a los aspectos citológicos, se discuten varios puntos taxonómicos, en cada grupo de número cromosomático diferente.

Lymnaea (4 subespecies), *Stagnicola* (15 especies y subespecies), *Acella* (1 especie), *Pseudosuccinea* (1 especie), *Bulimnea* (1 especie) tienen todas el número cromosomático haploid o de 18. No se encontraron diferencias morfológicas obvias, ni caracteristicas notables, para distinguir cariotipos entre o dentro de esos géneros. Tres especies de *Fossaria* llevan el número haploido de 18, pero *F. rustica* tiene 19. Esto sugiere que la última debería ser elevada al rango de especie, aunque muchos autores previos la consideraban como una subespecie de *F. modicella*. El par adicional de cromosomas es pequeño y con precuencia tiñe más bien débil durante la diakinesis espermatocita.

Radix (11 especies y subespecies) tienen 17 cromosomas haploidos. *"Lymnaea" natalensis* de Liberia también tiene tiene n=17, lo cual sugiere que es una especie de *Radix*, y su anatomía lo confirma. Tres especies tiene sólo 16(n) cromosomas: *"L." ollula* (=*viridis?*), *"L." tomentosa* y *"L." lessoni;* estas fueron incluídas en varios géneros nominales, principalmente por sus caracteristicas conchológicas. Un nuevo grupo, sin embargo, deberá denominarse para estas 16(n) especies.

Se observaron cromosomas somáticos en 5 especies (*Stagnicola palustris wyomingensis*, *S. exilis*, *S. catascopium*, *Bulimnea megasoma* y *Fossaria rustica*). Generalmente, todos los cromosomas de embriones en mitosis somática, eran de naturaleza meta- o submetacéntrica.

Los cromosomas pueden der de gran utilidad para el análisis de los cariotipos de limneidos, pero se necesitaran más estudios comparativos detallados, para sacar conclusiones seguras.

Se presenta una filogenia de los Lymnaeidae, considerada en base a la información citotaxonómica, citológica y paleontólogica.

АБСТРАКТ

ЦИТОТАКСОНОМИЧЕСКОЕ ИССЛЕДОВАНИЕ МОЛЛЮСКОВ-ЛИМНЕИД

АКИХИКО ИНАБА

Хромо с омы 16 видов моллюсков лимнеид из 22 пунктов наблюдалиcь как втечение сперматогенеза, так и во время овогенеза и митотического деления. В статье впервые приводятся данные о хромосомах 7 видов и подвидов, а также излагаются новые данные о хромосомах в сперматогониях, ооцитах, полярных телах и соматических митозах. Найденные числа хромосом сравниваются с теми, данные о которых приводились ранее.

В настоящей статье приводятся данные о хромосомных числах у 41 вида и подвида **Lymnaeidae**, относящихся к 7 родам. Обсуждаются различные таксономические моменты, основанные на цитологических данных по различным группам моллюсков с различным числом хромосом.

Lymnaea (4 подвида), ***Stagnicola*** (15 видов и подвидов), ***Acella*** (1 вид), ***Pseudosuccinea*** (1 вид) и ***Bulimnea*** (1 вид) - все имели гаплоидное число хромосом 18.

Не было обнаружено никаких морфологических различий или заметных характерных черт, достаточных, чтобы различить кариотипы среди или внутри этих родов. Три вида ***Fossaria*** имеют 18 гаплоидных хромосом, но ***F. rustica*** имеет 19. Это дает основание думать, что последний должен быть поднят до ранга вида, хотя многие авторы в прошлом рассматривали его как форму или подвид ***F. modicella***. Дополнительная пара хромосом малà и часто довольно слабо окрашивается при сперматоцитном диакинезе.

Radix (11 видов и подвидов) имеет 17 гаплоидных хромосом, ***"Lymnaea" natalensis*** из Либерии также имеет **n**= 17, что позволяет думать, что она тоже относится к ***Radix***, что подтверждает её анатомическое строение. 3 вида лимнеид имеют только по 16 хромосом: 4 ***"L." ollula*** (=***viridis?***), ***"L." tomentosa*** и ***"L." lessoni***. Они были включены номинально в различные родовые группы, главным образом по их морфологическим признакам. Однако, следует установить новые групповые названия для этих видов с 16 хромосомами.

Соматические хромосомы наблюдались у 5 видов (*Stagnicola palustris wyomingensis, S. exilis, S. catascopium, Bulimnea megasoma* и *Fossaria rustica*). В общем, все хромосомы в соматических митозах молодых эмбрионов были мета-или субметацентрическими по своей природе. Эти исследования хромосом могут быть очень полезными при анализе кариотипа лимнеид, но для получения более достоверных данных необходимы более детальные сравнительные наблюдения.

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THE ARTERIAL SYSTEM OF *BIOMPHALARIA GLABRATA* (SAY)[1]

Paul F. Basch[2]

ABSTRACT

A method for visualizing arteries of snails by injection with India ink is described. The arterial system of the planorbid *Biomphalaria glabrata* is illustrated with 28 figures. There are 2 aortic trunks: the posterior aorta serves the intestine and digestive gland area and ends as the gonadal artery in the ovotestis; the anterior aorta first gives off the cecal axis, renal artery, and smaller vessels to the anterior reproductive glands and columellar muscle, ending at the buccal vascular arborescence. From this point, near the circumesophageal ganglia, the buccal, pedal, and paired tentacular arteries arise to distribute hemolymph to the cephalopedal area. The pattern of capillary branching is generally irregularly dichotomous, lacking anastomoses and true capillary networks.

INTRODUCTION

The purpose of this paper is to illustrate and describe the main features of the arterial system of the planorbid snail *Biomphalaria glabrata* (Say). Because *Biomphalaria* species are important in Africa and South America as intermediate hosts of the human parasite *Schistosoma mansoni* Sambon, many publications have appeared describing their morphology and biology. However, the gross arterial system of *B. glabrata* has not been delineated, although Pan (1958) described certain histological details. The current investigation was undertaken in conjunction with various parasitological studies dealing with echinostome and other trematode larvae utilizing *B. glabrata* as first intermediate host. Sporocysts of such species as *Echinostoma barbosai* Lie & Basch, *E. paraensei* Lie & Basch, and *E. lindoense* Sandground & Bonne characteristically develop in the heart, and dispersal of rediae may be accomplished, at least in part, via the arteries. Moreover, infection with *S. mansoni* may result in damage to the snail's arterial walls, as discussed by Pan (1965). He reported thickened walls, hypertrophic and hyperplastic lining cells, and other changes from the normal histological condition. Among studies of the circulatory system of Basommatophora, the most extensive is that of Carriker (1946), who injected the arteries of *Lymnaea stagnalis appressa* Say with Emery's aqueous carmine solution, identifying and describing about 50 distinct arteries. Boer & Lever (1959) presented a diagram of major arteries of the ancylid *Ferrissia shimekii* Pilsbry (=*F. fragilis* var. *shimekii*, *fide* Basch, 1963). They did not specify the techniques used to work out details of arterial branchings; presumably they constructed the system from serial sections.

The technique employed in the present study is simple and rapid, requiring no narcotization of the snail and no special

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dyes or chemicals.

1. Materials required:

Binocular dissecting microscope;
Glass pipettes or medicine droppers drawn to a fine point in a flame and fitted with a rubber bulb;
Several petri dishes or similar containers;
A flat-bottomed clear glass shell vial, about 25 mm in diameter and 50 mm tall;
A beaker of about 300 to 500 ml capacity, filled with water, heated over a hotplate, gas or alcohol flame to boiling or a somewhat lower temperature as desired (see below);
Paper tissue;
India ink;
Fine watchmaker's forceps, two pairs;
A petri dish or similar container used as a dissecting pan. The bottom of the dish should be covered with wax, preferably black dissecting pan wax, available from biological supply houses;
Fine entomological pins, preferably "minuten nadeln."

2. Procedure:

The snail to be dissected is removed from the aquarium and left to dry in the air for a few minutes. The shell is blotted and dried with paper tissue and placed right side up (clockwise) in the bottom of a dry petri dish on the stage of the microscope. A pipette partially filled with India ink should be placed in a convenient location for quick use. The flat-bottomed vial is the most useful implement for cracking the snail shell. By observing through the microscope to the bottom of the empty vial, meanwhile applying pressure on the snail shell, it is possible to control shell breakage so that only the region over the heart is cracked. Fragments of shell are removed with the forceps and the pericardium is exposed. Using two forceps, open the pericardium to reveal the heart chambers. Grasp the ventricle and introduce the tip of the glass pipette through the ventricle wall and into the common aorta if possible. Inject the ink with moderate pressure on the rubber bulb, and immediately plunge the snail into the hot water for a few seconds in order to kill it in a softened condition. Some practice is necessary in order to find the proper pressure to fill the arteries with ink without producing spillage into the venous sinuses; even so, some specimens may be imperfectly injected and of only limited utility. If the hot water bath is boiling, the hemolymph in the venous sinuses will coagulate into a pink solid mass. Regulation of the water temperature will result in fluid or coagulated hemolymph, as desired.

After injection and the hot water bath, the snail is placed in a clean petri dish and the remainder of the shell removed. Mucus and excess ink may be cleaned off, and the snail is ready for pinning in the wax-bottomed dish for dissection under water.

The snails used in this study were mature albino *Biomphalaria glabrata* from 20 to 25 mm in diameter. All were laboratory-raised and free from trematode infection.

RESULTS

Results of the India ink injection of arteries vary somewhat, depending on technique and the idiosyncracies of individual specimens. The general outline of the arterial system of *Biomphalaria glabrata* is illustrated in Fig. 28. Some departure from this precise pattern may be expected in the smaller branches, but the main outlines and larger branches were invariable in several dozen specimens of the population studied.

Since the principal intent of this paper is to illustrate the arterial system

of *Biomphalaria glabrata* (Figs. 1-28), the verbal description will be brief.

The ventricle leads directly into a short, broad, common aorta from which a smaller lateral branch passes to the adjacent albumen gland. The albumen gland artery branches to the gland and also supplies a portion of the pyloric region and proximal loop of the intestine (Fig. 27). This artery is easily broken and not shown on the photographs.

The common aorta continues a short distance caudad and bifurcates to form the anterior and posterior aortae (Figs. 12, 27, 28) in the same manner as that illustrated by Boer & Lever (1959) for *Ferrissia*. The posterior is the simpler of the two aortae, giving rise first to a few branches that serve the intestine and digestive gland area. After passing through the digestive gland (Fig. 17), the posterior aorta supplies the seminal vesicle and continues, as the gonadal artery, along the columellar surface of the posterior portion of the digestive gland to the ovotestis (Fig. 16).

The anterior aorta gives rise to a large artery, here termed the cecal axis, immediately after passing through the proximal loop of the intestine (Figs. 9, 10, 12, 27, 28). Branches of the cecal axis serve the cecum and other portions of the digestive system, including the esophagus. The stem leading to the esophagus divides immediately into several long, parallel vessels, the esophageal arteries, running anteriad along the surface of that organ (Figs. 22, 28). Other organs served via the cecal axis include the anterior apex of the digestive gland, the rectum and columellar muscle (Figs. 14, 17) and the hermaphroditic duct.

The anterior aorta continues anteriad, next giving off a blunt, short branch (Fig. 12) that traverses the area near the inner end of the pulmonary cavity and opens into a sinusoidal complex ramifying into the blood spaces within the folds of the saccular portion of the kidney. Further anteriad, the anterior aorta gives off a small branch to the columellar muscle and then passes through the connective tissue parallel to the rectum, near the base of the rectal ridge. In this area various fine, short, lateral branches pass to the anterior reproductive gland complex, including the oviduct, nidamental gland and uterus (Fig. 13). The anteriormost of this series of branches is usually larger than the others and ramifies over the surface of the spermatheca (Fig. 24). Smaller arteries in this region also go to the columellar muscle and posterior part of the foot. From approximately this point forward the anterior aorta runs free and unbranching within the cephalic hemocoel until it reaches the level of the circumesophageal ganglia. Five major arterial stems meet at this point, termed the buccal vascular arborescence (Figs. 6, 28), following Carriker (1946). These vessels are: a) the anterior aorta, carrying blood forward; b) the left and right tentacular arteries, giving rise also to the velar arteries bilaterally and the preputial artery on the left side only; c) the buccal artery, medial anterior continuation of the anterior aorta, which expands into a dilated bulb (Figs. 5, 6); d) the common pedal artery, directed ventrad into the pedal musculature where it divides immediately into anterior and posterior pedal arteries (Fig. 7). In addition to these major arteries, smaller vessels pass directly to capillaries and sinusoidal chambers in the circumesophageal ganglia. The arterial supply to the ganglia was not worked out in detail in the present study.

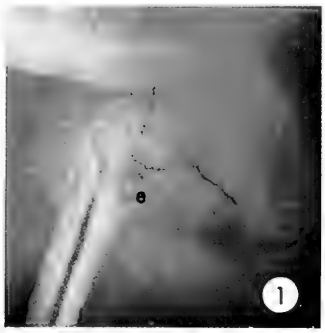
The arterial system ends in capillaries everywhere except in the renal sinusoids, bulb of the buccal artery, and possibly around the circumesophageal ganglia. Figures 19 to 26 show various patterns of the ramification of smaller arteries in different organs. Typically the pattern of branching is irregularly dichotomous (Figs. 19, 24). True capil-

FIGS. 1-9. Injected arteries of *Biomphalaria glabrata*. FIG. 1. External view of undissected specimen showing base of right tentacle, with tentacular artery and branches. FIG. 2. Same, with mantle collar removed. FIG. 3. Cephalic area showing portions of right and left tentacular arteries and preputial artery. FIG. 4. Further dissection of cephalic area with esophagus removed and preputium turned to reveal anterior aorta and preputial artery. FIG. 5. Further dissection of cephalic area. The buccal mass has been pulled forward to show the buccal bulb. Note branching of right velar and tentacular arteries. FIG. 6. Complete dissection of arteries of the central cephalic area, showing the buccal vascular arborescence. FIG. 7. Dissection of foot musculature showing primarily the branches of the posterior pedal artery. FIG. 8. The ventricle and major branches of the arterial system with major organs *in situ*. Pericardium and atrium removed. The dark mass to the right of the ventricle is a portion of the proximal kidney. FIG. 9. Same specimen as Fig. 8, digestive gland removed.

FIGS. 10-18. Injected arteries of *Biomphalaria glabrata*. FIG. 10. Arterial distribution to the cecum and proximal intestine area. FIG. 11. Same specimen as Fig. 10, showing branching of small arterial capillaries on the digestive gland. FIG. 12. Major arterial branches; intestine cut at "i." FIG. 13. Portion of anterior aorta showing arterial supply to the anterior reproductive glands. FIG. 14. Cecal axis and major branches. FIG. 15. Same specimen as Fig. 14, dissected further to show pattern of smaller branches. FIG. 16. Gonadal artery and branches. Undissected specimen. FIG. 17. Portion of the posterior aorta. Most of the digestive gland has been removed. FIG. 18. Same specimen as Fig. 17, showing arterial distribution to the seminal vesicle area.

Key to Lettering, Figures 1 - 18.

ag	albumen gland	hd	hermaphroditic duct
bm	buccal mass	i	intestine
c	cecum	ng	nidamental gland
cg	circumesophageal ganglia	p	preputium
dg	digestive gland	rr	rectal ridge
e	eye	sv	seminal vesicle
es	esophagus	v	ventricle
g	gizzard		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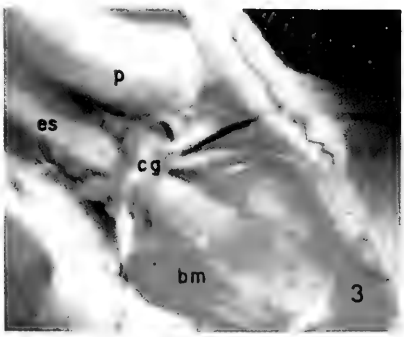
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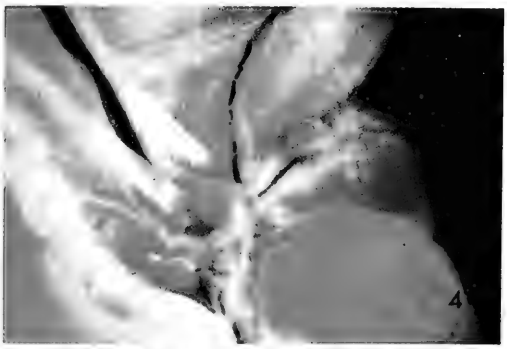
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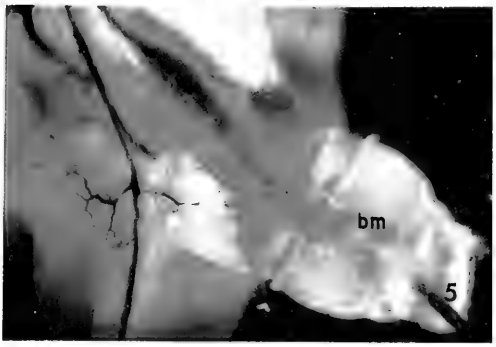
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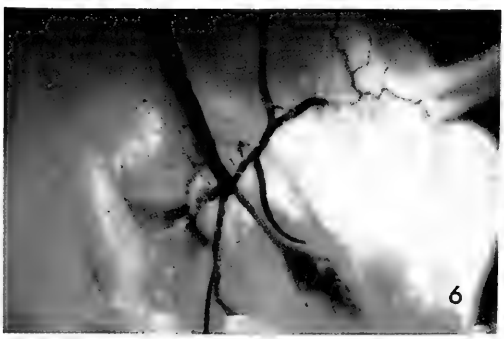
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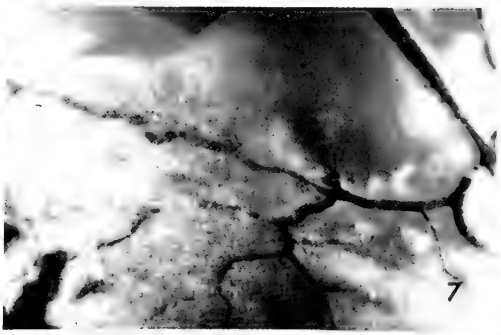
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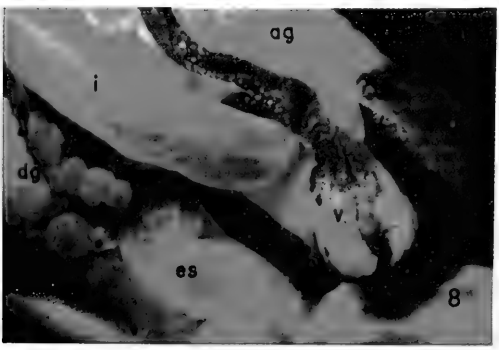
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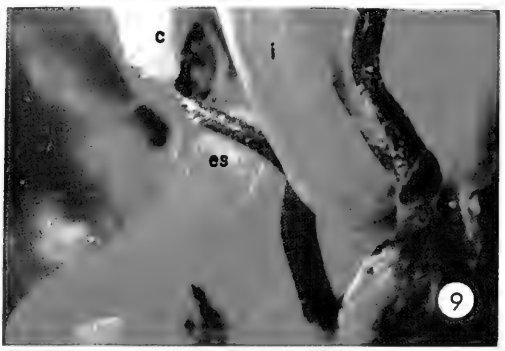
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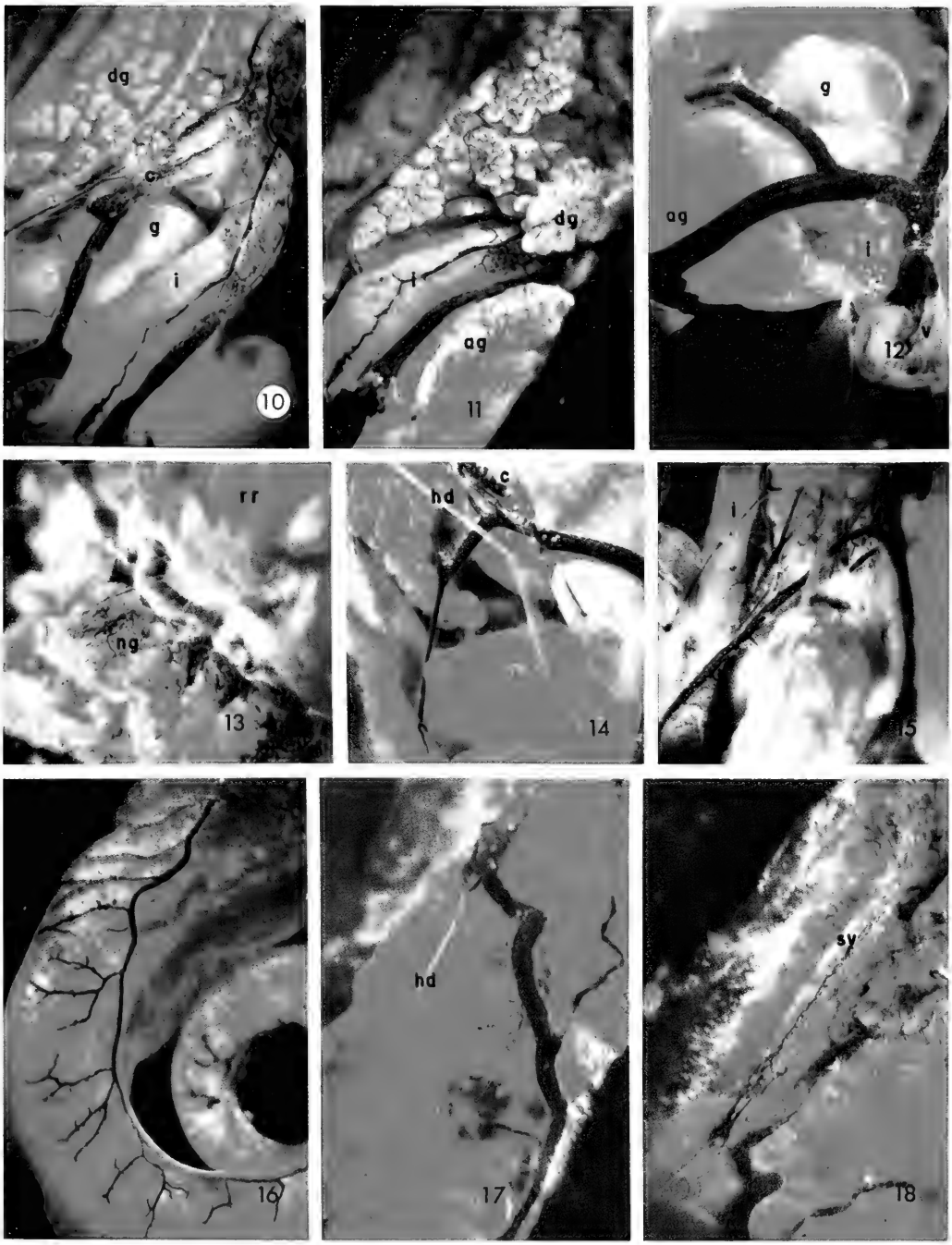
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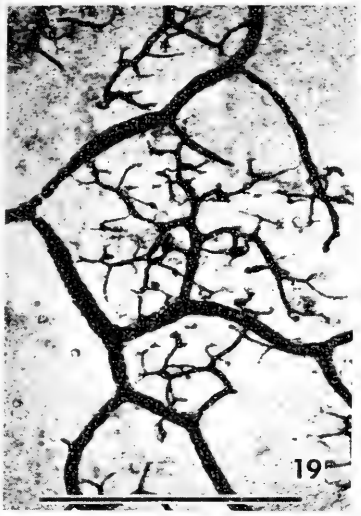

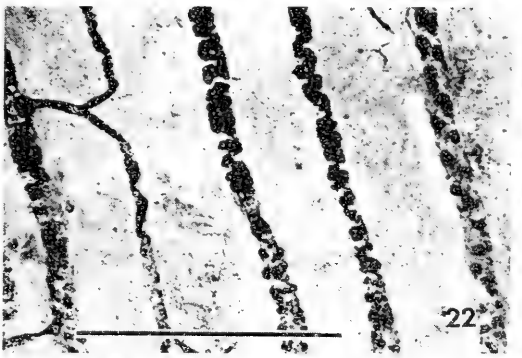

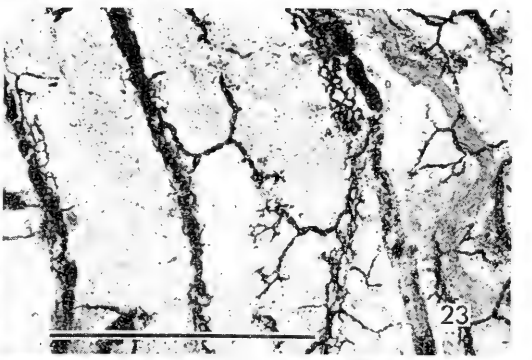

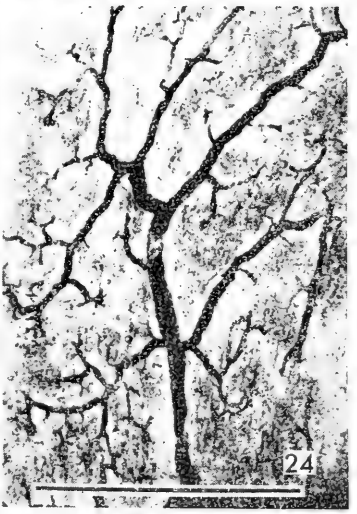

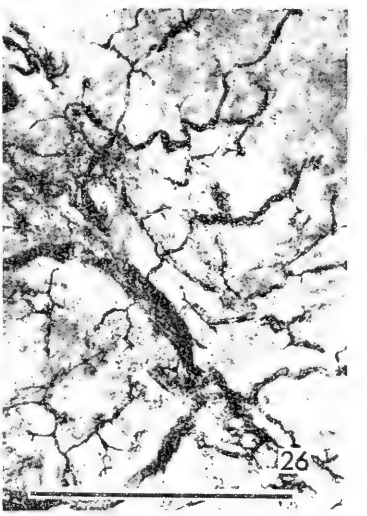

FIGS. 19-26. Injected arteries of *Biomphalaria glabrata*. Unfixed wet-mount preparations from a freshly injected specimen to show patterns of branching. FIGS. 19-21. Flattened surface of proximal loop of intestine. Scale lines: Fig. 19, 1 mm; Figs. 20, 21, 100 u. FIGS. 22, 23. Flattened surface of esophagus near its entrance to gizzard. Scale lines, 1 mm. FIG. 24. Flattened surface of spermatheca. Scale line, 1 mm. FIG. 25. Portion of circumesophageal ganglia. The arrow indicates the statocyst containing otoliths. Scale line, 100 u. FIG. 26. Flattened surface of nidamental gland. Scale line, 1 mm.

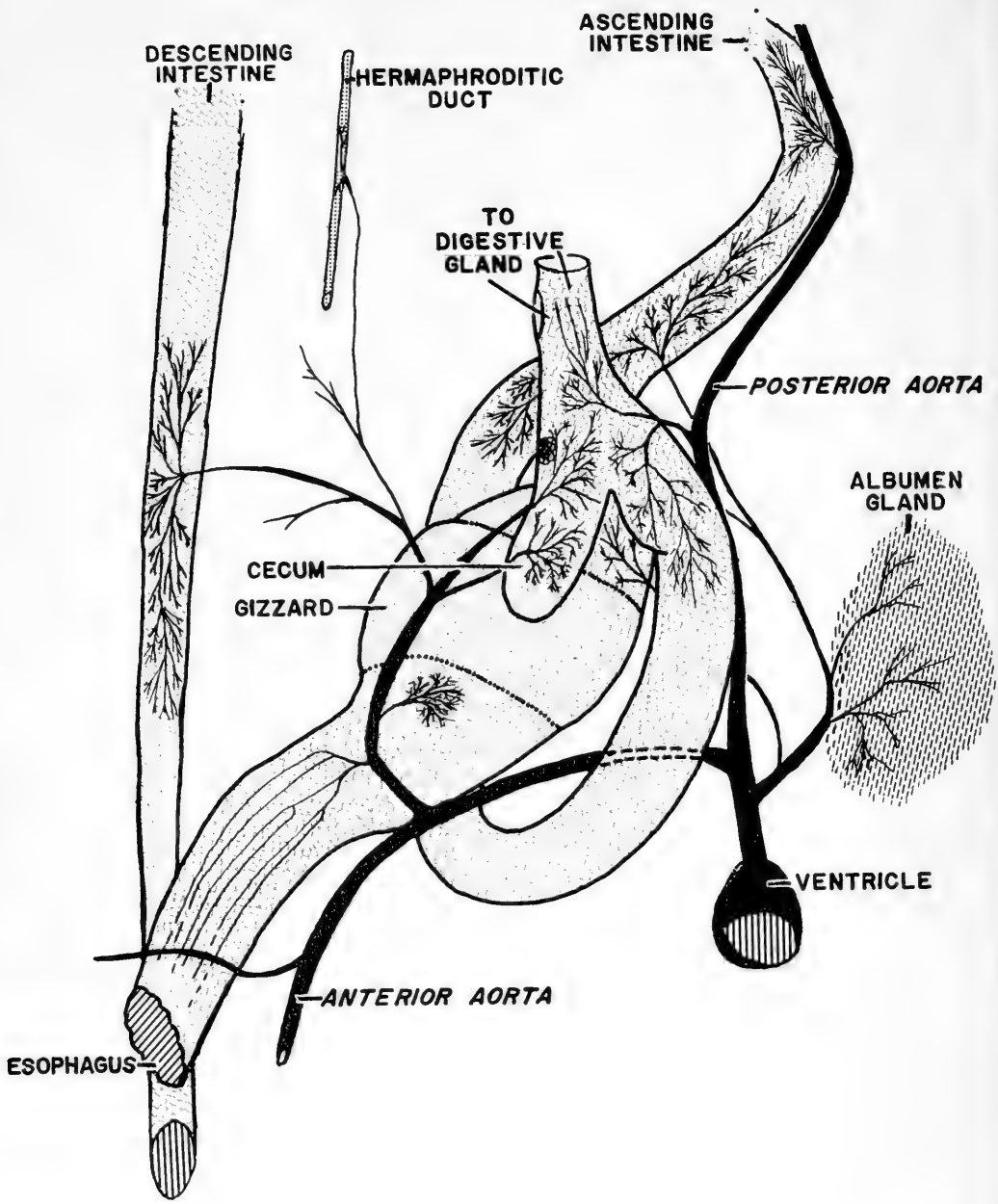

FIG. 27. A portion of the digestive system of *Biomphalaria glabrata*, showing the typical pattern of arterial distribution.

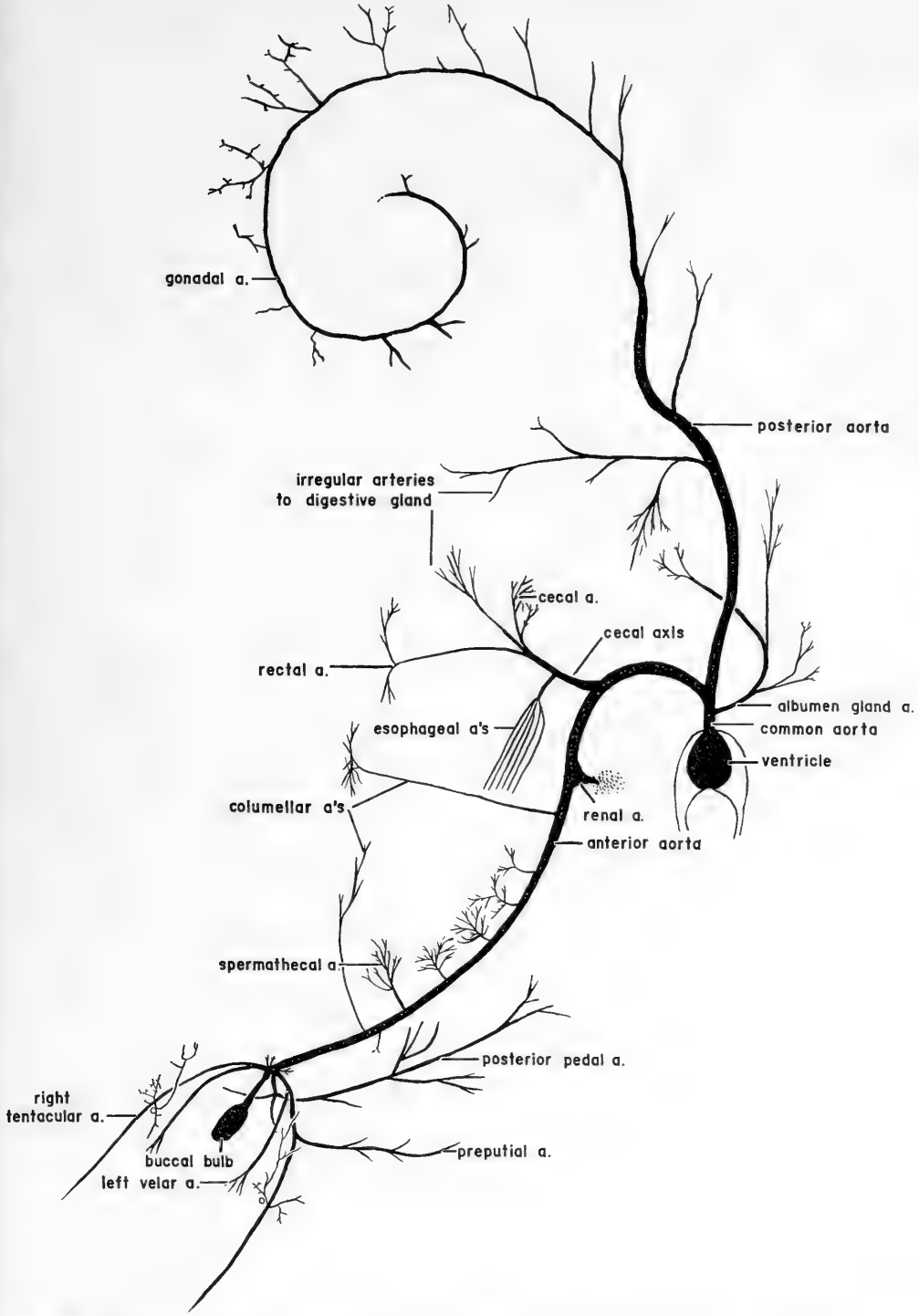

FIG. 28. Arterial system of *Biomphalaria glabrata*. Semidiagrammatic illustration of primary branches of the entire arterial system.

lary networks consisting of numerous anastomoses were not found, although occasional cross-connections appear between the smallest branches. The finer capillaries are much as those described in *Lymnaea stagnalis appressa* by Carriker (1946). Fig. 21 shows extravasation of injected ink, presumably through the "capillary ostioles" of Carriker.

DISCUSSION

The scanty literature on pulmonate circulatory systems has recently been reviewed by Hyman (1967), who refers to several older papers, primarily on Stylommatophora. The most extensive of these, by Polinski (1929), deals with a comparative study of arteries of several families of land snails, without mention of Basommatophora. Patterns of arterial distribution in Helicidae and other Stylommatophoran families appear to be quite different from those found in *Biomphalaria*. The thorough and detailed investigation of the arteries in *Lymnaea stagnalis appressa* by Carriker (1946) can serve as a model of the type of studies needed before a discussion of homologies in the circulatory system of Basommatophora can be undertaken. The general pattern of arterial distribution appears, however, to be rather similar in *Lymnaea*, *Ferrissia*, and *Biomphalaria*, particularly in the latter two. Through use of the simple technique described here, comparative studies of different groups of Basommatophora can be readily undertaken.

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RÉSUMÉ

LE SYSTÈME ARTERIEL DE *BIOMPHALARIA GLABRATA* (SAY)

P. F. Basch

Une méthode pour rendre visible les artères des mollusques par injection d'encre de Chine, est décrite. Le système artériel du planorbe *Biomphalaria glabrata* est illustré par 28 figures. Il y a 2 troncs aortiques: l'aorte postérieure irrigue l'intestin et la zone des glandes digestives et se termine comme artère gonadique dans l'ovotestis; l'aorte antérieure donne d'abord naissance au tronc coecal, à l'artère rénale et à de plus petits vaisseaux des glandes reproductrices antérieures et du muscle columellaire, puis se termine en arborescence vasculaire buccale. A partir de là, près des ganglions circumoesophagiens, apparaissent les artères buccale, pédieuse et tentaculaires qui distribuent l'hémolymphe dans la région céphalopédieuse. La structure des ramifications des capillaires est en général grossièrement dichotomique, manquant d'anastomoses et d'un véritable réseau capillaire.

RESUMEN

EL SISTEMA ARTERIAL DE *BIOMPHALARIA GLABRATA* (SAY)

P. F. Basch

Se describe un método para visualizar las arterias de caracoles inyectando tinta china. El sistema arterial de *Biomphalaria glabrata* se ilustra con 28 figuras. Existen dos troncos aórticos: la aorta posterior sirve al área de las glándulas digestivas e intestinales y termina como la arteria gonadal en el ovotestis; la aorta anterior primero se separa del eje cecal, arteria renal y pequeños vasos anteriores a las glándulas reproductoras y músculo columelar, para terminar en la ramificación vascular bucal. Desde este punto, cerca del ganglio circumesofágico, las arterias bucales, pedales y tentaculares, surgen para distribuir la hemolinfa al área cefalopedal. El sistema de ramas capilares es general e irregularmente dicotómico, faltando anastomosis y verdaderas redes capilares.

АБСТРАКТ

АРТЕРИАЛЬНАЯ СИСТЕМА У *BIOMPHALARIA GLABRATA* (SAY)

П.Ф.БАШ

Описывается метод инъекции китайской туши в артерии улиток, чтобы сделать их хорошо заметными. Описывается и иллюстрируется 28 рисунками артериальная система планорбииды ***Biomphalaria glabrata***. Имеется два ствола аорты- задняя аорта обслуживает кишечник и пищеварительную железу, заканчиваясь ввиде гонадной артерии в гермафродитной железе; передняя аорта дает слепой вырост, почечную артерию и более мелкие сосуды, идущие вперед, к половым железам и колюмеллярному мускулу, заканчиваясь буккальным сосудистым разветвлением. Отсюда близ окологлоточного ганглия отходят буккальная ножная и парные тентакулярные артерии, по которым гемолимфа поступает в цефалопедальную область. Части капиллярных разветвлений обычно неправильно-дихотомичные, анастомозы и настоящая капиллярная сеть сосудов отсутсвует.

MALACOLOGIA, 1969, 7(2-3): 183-209

STUDIES ON "TROPICORBID" SNAILS (*BIOMPHALARIA:* PLANORBIDAE) FROM THE CARIBBEAN AND GULF OF MEXICO AREAS, INCLUDING THE SOUTHERN UNITED STATES[1,2]

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ABSTRACT

A large number of planorbid "species" have been reported from the neotropics. Among these are forms comprising natural or experimental intermediate hosts of *Schistosoma mansoni,* which until recently have been assigned to the genus *Tropicorbis*. Because their systematics are still largely unstable, an attempt has been made to investigate a number of these forms from the Gulf of Mexico and Caribbean area, utilising shell features and, for some populations, also morphology of genitalia to check on identity, evaluate synonymy and to determine groupings. For this purpose tropicorbid samples were collected by the author or obtained from colleagues from a variety of locations in this area and museum collections were also examined. To conform with Opinion 735 of the International Commission of Zoological Nomenclature, the name now used for these snails is *Biomphalaria*.

The study has shown that the nominal species on record for the area could now be consolidated into 8 species: *Biomphalaria obstructa, B. havanensis, B. pallida, B. fieldii, B. riisei, B. straminea, B. albicans* and *B. schrammi*. The first 6 species are here considered to be more closely related to the South American *B. peregrina*. The shell was not found to be a reliable tool in differentiating the first 4 species from one another, i.e., *B. obstructa, B. havanensis, B. pallida* and *B. fieldii,* but that it could be used to differentiate them from the other 4 species, i.e., *B. riisei, B. straminea, B. albicans* and *B. schrammi,* each of which is conchologically distinct. The genitalia provided the following criteria of interspecific diagnostic value: presence or absence of a vaginal pouch, resp. of vaginal corrugations or foldings; length of the prostate gland; length ratios of the female tract (from the point of bifurcation of the hermaphrodite duct to the female genital opening) to the following organs: penial complex; hermaphrodite duct; spermathecal duct and sac; and prostate; also length ratio of preputium to vergic sac.

INTRODUCTION

The stabilization of the classification and nomenclature of the neotropical planorbid snails has become increasingly important because certain species in the neotropical area act as intermediate hosts of the medically important schistosomes, while others are potential hosts for these flukes. Over 250 species have been named from the neotropics between 1789 and 1957 (Harry, 1962). This number includes the helisomes and the drepanotrematids

[1]This investigation was supported by a Public Health Service Research Career Award (no. K6-AI-18, 424) and by research grant number AI-02898, from the National Institute of Allergy and Infectious Diseases.

[2]Based in part on a paper read at the First International Congress of Parasitology, Rome, September, 1964.

which are not dealt with in the present study. Generally, the systematics of these planorbids are still in a chaotic state; not only has the validity of certain species often been disputed but also to which of several genera it should be assigned. Many of the species and genera are not well defined, still others are nomina nuda.

The generic names for the planorbid group here considered that are commonly found in the literature from the 1930's onward are the relatively recent names *Australorbis* and *Tropicorbis* for the larger and the smaller forms, respectively. As it became increasingly clear that these and other 'genera' were too similar to warrant generic distinction, authors tended to revive older valid names or to retain *Australorbis*.

A recent ruling of the International Commission on Zoological Nomenclature (Opinion 735, 1965) directed that the generic name *Biomphalaria*, established for African forms, be given precedence for all genera considered to be synonymous (see Systematic Review). For this reason the name *Biomphalaria* will be used in this paper while retaining the designation "tropicorbid" to indicate which group of the species is discussed.

It is the purpose of the present study to present certain morphological characteristics thought to be of significance in the differentiation of species traditionally placed under *Tropicorbis*, and to group together, or to bring into synonymy, some of the nominal species which after examination proved to have been described only on the basis of intraspecific variation.

Special emphasis has been placed in this study on the tropicorbids found in the region of the United States bordering the Gulf of Mexico which is the northernmost zone of distribution of these neotropical snails. Certain forms occur in southern Florida, central and southern Louisiana, and in central, southern and southwestern Texas. In previous studies these planorbids have been assigned to the species *Tropicorbis havanensis* (Pfeiffer), *T. obstructus* (Morelet), and *T. obstructus donbilli* (Tristram). Baker (1945), Berry (1947) and Brooks (1953) reported observations on the morphology of tropicorbid snails from Louisiana; Berry (1947) and Malek (1952, 1962) reported on collections from Texas. In the present study the morphology of the tropicorbids found in several localities of the Gulf of Mexico States (of the United States) was further investigated and compared with that of related species in neighbouring regions of the Gulf of Mexico and the Caribbean and with a species from Brazil. Information was also obtained on the systematic status, distribution and ecology of these snails.

MATERIAL AND METHODS

A number of tropicorbid snails from the southern United States (Louisiana) and neighbouring mainland areas (Mexico, British Honduras and Panama) were collected by the writer; others, from Texas, U.S.A., and also from Jamaica, Costa Rica and Venezuela were kindly contributed alive by colleagues, while other colleagues contributed fixed material from Florida, U.S.A., from the Dominican Republic and Puerto Rico (Fig. 1a). A number of shells of various forms were examined in the collections of the Museum of Zoology, University of Michigan, the Pan American Health Organization Snail Identification Center at the Instituto Nacional de Endemias Rurais, Belo Horizonte, Brazil, and the U.S. National Museum, Washington, D.C.

A number of generations of the majority of the field-collected samples, and those contributed alive by colleagues were reared in the laboratory for further study. Aquaria containing filtered water and aquatic plants with broad leaves provided excellent culture media for the tropicorbids. White enamel coated pans with aerated water were also used satisfactorily.

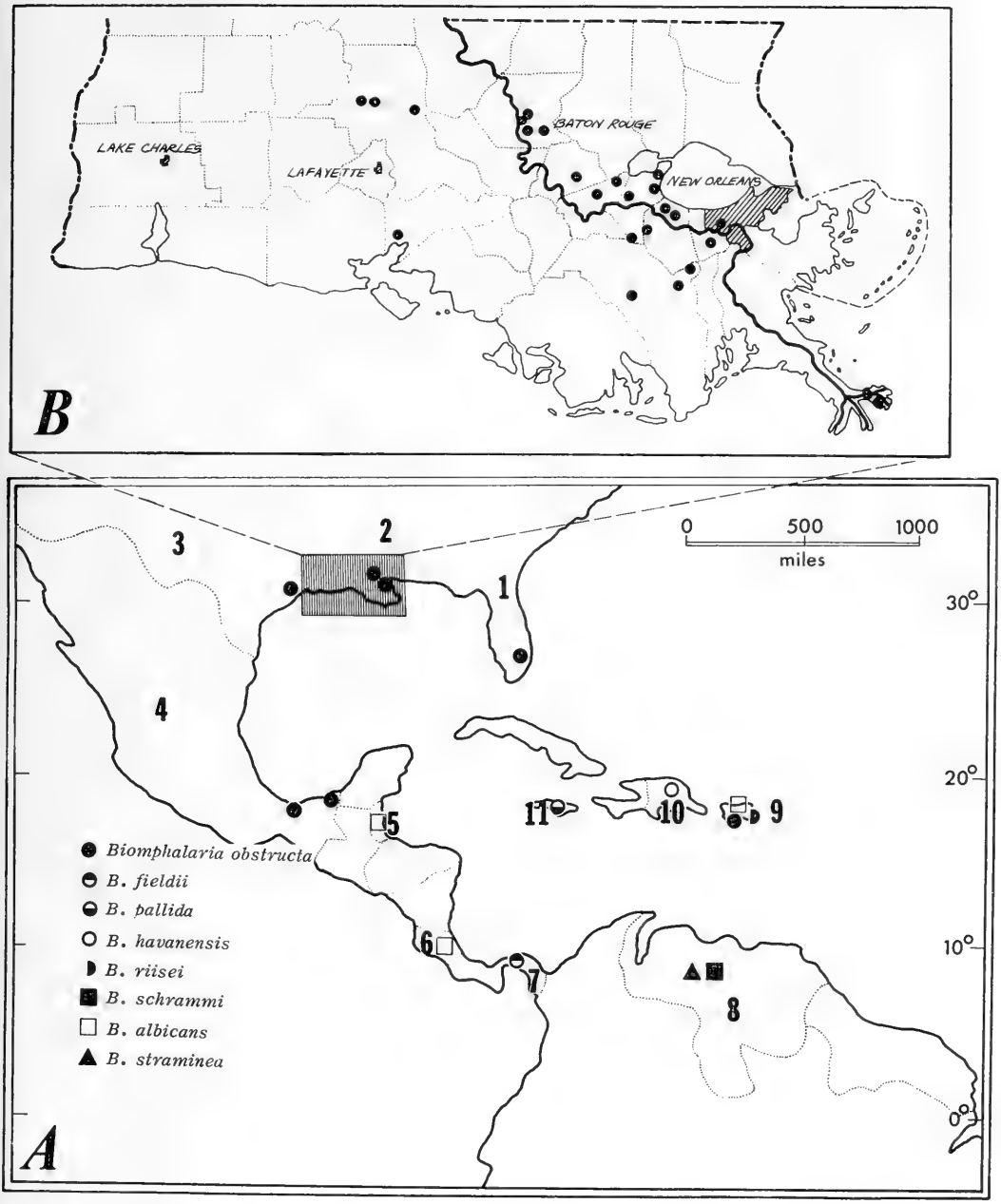

FIG. 1. Origin of tropicorbids examined. **a.** Distribution of species of *Biomphalaria* recognized in the Gulf of Mexico and Caribbean area; 1. Florida; 2. Louisiana; 3. Texas; 4. Mexico; 5. British Honduras; 6. Costa Rica; 7. Panama; 8. Venezuela; 9. Puerto Rico; 10. Dominican Republic; 11. Jamaica. **b.** Distribution of *Biomphalaria obstructa* in Louisiana, U.S.A.

For anatomical studies the snails were anaesthetized with menthol or killed by gradual immersion in hot water, and the animals were then pulled out of the shells. They were examined morphologically in water or after fixation in alcohol.

Water samples from natural habitats were collected and pH determinations were made with La Motte standards and indicators in the field, and with a Beckman 'Zeromatic' meter in the laboratory. Salinity was determined with a 'Solu Bridge' salinity meter.

SYSTEMATIC REVIEW

Most of the numerous nominal neotropical planorbid forms were originally assigned to the all-embracing genus *Planorbis*, a taxon which, as presently understood, is not represented in this geographical region, and were only later assigned to a number of other genera. As generally accepted at the present time, these neotropical forms certainly fall into 2 generically distinct categories[3]: the genus *Drepanotrema* which does not concern us here, and a multitude of forms comprising actual or potential hosts of *Schistosoma mansoni*, now all considered to be congeneric, which comprise the 'tropicorbid' group dealt with in this paper. Other genera may be discerned in the future.

Among the genera that have been available for accomodating the neotropical species now usually considered to be congeneric are: *Tropicorbis* Pilsbry & Brown 1914; *Australorbis* Pilsbry 1934; *Taphius* H. A. Adams 1855; *Platytaphius* Pilsbry 1924; *Armigerus* Clessin 1884 (by designation by Morrison, 1947); and *Planorbina* Haldeman 1842 (by designation by Dall, 1905). *Armigerus* had previously and justifiably been placed under *"Tropicorbis"* by Pilsbry (1934) and Baker (1945) and the generic concept of *Platytaphius* is also generally considered as superfluous (Hubendick, 1955; Harry, 1962).

That there is a close relation between the medically important species that have been assigned to *Australorbis*, *Tropicorbis* and to the African *Biomphalaria* Preston 1910, has been recognised by various workers (Pilsbry, 1934; Baker, 1945; Hubendick, 1955). My morphological studies (1952) on *Biomphalaria glabrata* (*Australorbis glabratus*), of several species of African *Biomphalaria* (1954, 1958) and of *B. obstructa* (*Tropicorbis obstructus* (1952, 1962) also support the conclusion that differentiation of the African and neotropical forms on the generic level was not warranted.

The instability of the nomenclature of this group is reflected in the publications of the last decade. Thus, in the late 1950's Paraense and his co-workers replaced the names *Australorbis*, resp. *Tropicorbis* by *Taphius* (Paraense & Deslandes 1957a, b; 1958), but later reverted to *Australorbis* for all actual and potential intermediate hosts of *S. mansoni* and related forms in the neotropics (Paraense, 1963; Paraense & Deslandes, 1962; Paraense et al., 1964), while Hubendick (1961) and Harry & Hubendick (1964) continued to favor *Taphius*, and others, e.g., Baker (1960) and Burch (1960), followed Walker (1917), Germain (1921) and Thiele (1931) in using the valid name, *Planorbina*, which had priority over all other known names.

In an attempt to stabilize the nomenclature of this group the subject was discussed by Barbosa, et al. (1961). A case in favor of giving priority to the genus *Biomphalaria* was submitted by Dr. C. A. Wright (1962) to the International Commission on Zoological Nomenclature and published in the Bulletin of Zoological Nomenclature. The application was supported by some and argued against by other specialists. Finally, a recent ruling by the Commission (Opinion 735, 1965) directed

[3]The genus *Helisoma*, whose distribution also extends into the neotropics, is nearctic.

that, to replace *Australorbis* (including *Tropicorbis*), the generic name *Biomphalaria* be given precedence over the earlier generic names (*Planorbina*, *Taphius*, *Armigerus*) by any zoologist who considered that any or all of these names applied to the same taxonomic genus. The 4 genera (*Biomphalaria*, *Planorbina*, *Taphius* and *Armigerus*) have been placed on the Official List of Generic Names in Zoology, and their respective type species (*smithi* Preston 1910; *olivaceus*, Spix 1827; *andecolus*, d'Orbigny 1835, and *albicans* Pfeiffer 1839) are also placed on the Official List of Specific Names in Zoology. The ruling of the Commission specifies that if any zoologist considered the type-species of any of the 3 above neotropical genera to be congeneric with *Biomphalaria*, their generic names could not be used by him in preference to *Biomphalaria*. The cautious wording of the Opinion arose from the fact that the synonymy of some forms diagnosed as *Taphius* has been disputed (Walter, 1963) and that this name should remain accessible.

Because I consider the species *smithi*, *olivaceous* and *albicans* congeneric, I am giving precedence to *Biomphalaria*. For the history of the genera *Planorbina* and *Armigerus* the reader is referred to Barbosa et al. (1961). As to the genus *Tropicorbis*, its history, and its distinction from *Australorbis*, which is represented in the same faunal region, deserve some comments. Pilsbry & Brown (1914) proposed *Tropicorbis*, without any diagnosis, as a section of *Planorbis*. They designated *P. liebmanni* Dunker as its type species. Germain (1921) considered it a subgenus of *Planorbis*, incorrectly stating the type to be *P. maya* Morelet. H. B. Baker (1930) and later Pilsbry (1934) gave *Tropicorbis* generic status, and reported on the anatomy of *T. pallidus*. F. C. Baker (1945) placed *Tropicorbis* in the subfamily Planorbinae, and divided the genus into 3 subgenera: (1) *Tropicorbis* s.s., type *Planorbis orbiculus* Morelet. (2) *Obstructio* Haas 1939, type by original designation *Planorbis janeirensis* Clessin. (3) *Lateorbis* F. C. Baker 1945, type *Planorbis pallidus* C. B. Adams. The main basis on which the differentiation was made was the absence of apertural lamellae (subgenera *Tropicorbis* and *Lateorbis*), or their presence during one stage of the growth of the shell (subgenus *Obstructio*). Further, in *Lateorbis*, the shell whorls are rapidly increasing in diameter, while in *Tropicorbis* and *Obstructio* they increase slowly. Recent studies (Richards, 1963, in particular) on species referred to *Tropicorbis*, and also on *Australorbis*, have shown that the apertural lamellae are not a consistent characteristic on which to base differentiation. They might be present in some individual shells and absent in others in the same colony. Moreover, species of *'Tropicorbis'* as well as of *'Australorbis' glabratus* which normally do not possess apertural lamellae, might develop them when exposed to adverse environmental conditions such as drought. Lamellae did develop in some young laboratory-reared *Biomphalaria glabrata* in my aquaria when the snails were stranded for several days above the water level. Thus it seems that the division of *Tropicorbis* into these subgenera or any separation from *'Australorbis'* on the basis of lamellae is artificial.

Other differences described for *Tropicorbis* and *Australorbis* also cannot be considered of value for systematic division at the generic level. Pilsbry (1934) originally differentiated *Australorbis* and *Tropicorbis* on the basis of: differences in the spermathecal duct which is moderately developed in *Tropicorbis* and is extremely short in *Australorbis;* of the length ratio of vergic sac to preputium; of the marginal teeth of the radula; and of the size of the shell, which is 'rather large' in *Australorbis* and 'much smaller' in *Tropicorbis*. Among these characteristics, the ratio of the length of the vergic sac

to the preputium varies even among tropicorbid species. As to the size of the shell, it is known that *Australorbis* in certain habitats assumes a small size that is within the range of the tropicorbid species. For example, *Biomphalaria glabrata* from most habitats on the Antillean island of St. Lucia (Malek, unpublished data) are comparable in size to *B. riisei* from Puerto Rico. In fact, the only characteristics of diagnostic value in distinguishing *"Australorbis"* from *"Tropicorbis,"* though also not at the generic level, are: *Australorbis* has a longitudinal pigmented ridge on the ventral surface of the kidney of mature adults, or a pigmented outline in young individuals, that is absent in *Tropicorbis*. The ovotestis of species of *Australorbis* consists predominantly of branched acini, whereas that of *Tropicorbis* consists of simple unbranched ones. In other respects the species assigned to these 2 'genera' are similar and should undisputedly be regarded as congeneric.

OCCURRENCE OF *BIOMPHALARIA OBSTRUCTA* IN THE SOUTHERN UNITED STATES

1. Distribution

Louisiana: Figure 1b shows the localities in Louisiana where tropicorbids have been collected. It is evident that the snail is prevalent in the central, southern and southeastern parts of the state. Tropicorbids had been previously collected from 3 localities, in and close to New Orleans, and from Baton Rouge. They had been diagnosed as *Tropicorbis obstructus* except for the samples from Audubon Park, New Orleans, and from Baton Rouge which were labelled *T. havanensis* (F. C. Baker, 1945, and Berry, 1947, respectively).

During the present study tropicorbids were collected in a total of 24 sites in the following parishes (and localities): Plaquemine (2 localities in the Mississippi Delta at Pass à Loutre); La Fourche (Lake Penchak); Iberia (Avery Island); St. Charles (6 localities: Bonnet Carré Spillway, Clayton Pond, Willswood Pond, Bayou near Des Alemandes, 2 localities in Lake El-Salvador); Jefferson (Swamp along Highway 90 West); New Orleans (Lagoon in Audubon Park); St. John the Baptist (Borrow-pit along Highway 61, 2 localities in Bayous along Highway 51); Ascension (Bayou at Sorrento, 2 localities in Blind River); East Baton Rouge (small lake near auditorium on Louisiana State University campus, lake across from same auditorium, Capitol lake in Baton Rouge); St. Landry (Swamp at Krotz Springs, swamp along US Highway 190, swamp along Highway Louisiana 105).

The snails were not found, where looked for, north of Lake Pontchartrain, in the Pearl River drainage bordering the State of Mississippi, in the western part of the state bordering Texas, in Rapides and Avoyelles parishes, and in the northeastern part of the state in Tensas parish.

Florida and Texas: The snails from Florida were collected by Dr. Henry Leigh in Dade County in and near Miami. In Texas, Berry (1947) reported the following localities for *Tropicorbis obstructus* (*Biomphalaria obstructa*) and *T. obstructus donbilli* (*B. obstructa*): Bell County (Temple-Belton Highway near Temple); Bexar County (Woodlawn Lake, San Antonio); Callahan County (spring 3 miles east of Baird; Cameron County (Brownsville 4 miles east of city, in north of city and in Santa Maria); Concho County (Concho River at Paint Rock); Fort Bend County (lake east of Sugar Land); Hays County (San Marcos River, Federal Fish Hatchery); Hidalgo County (roadside ditch east of Hidalgo); Kinney and Val Verde Counties (Sycamore Creek); Maverick County (roadside ditch east of Quemade); Nolan County (lake southwest of Sweetwater); Runnels County (east of Ballinger); Travis County (Onion Creek, US Highway 81); Williamson County (Brush Creek between Round Rock and Ceder

Park); Zapata County (Lake on US Highway 83).

In the present study tropicorbid snails could not be found in eastern Texas adjacent to Louisiana.

Mississippi: The southern, central and southwestern parts of the state of Mississippi were surveyed in this investigation, but tropicorbids were not encountered.

2. Habitats

Louisiana: Tropicorbid snails occur in Louisiana in the freshwater marshes and bayous common in the southern part of the state. In the latter zone, they were also collected from ponds, lagoons and borrow-pits. In the Delta of the Mississippi River, the snails inhabit protected areas in the channels where they attach themselves to floating mats of water hyacinth and alligator grass. Away from the main channels in the Delta, the snails are found in permanent ponds rich in aquatic plants. Their distribution in Louisiana coincides with bottomland hardwoods, cypress and tupelo (*Nyssa*) as well as with the delta and subdelta mud flats and their freshwater marshes. At the margins of the latter 2 zones, seasonal high tides from the Gulf of Mexico result in the sporadic disappearance of all or most of the well-established colonies.

In general, the habitats of these snails in Louisiana are clear or slightly turbid waters with mud banks and humus bottoms. A slight increase in the salinity of the water is a limiting factor for their occurrence in coastal areas. The range of salinity in all the habitats examined was 0.01-0.055, expressed in % sodium chloride. The hydrogen ion concentration varied slightly from 7.6-8.0, but in certain microhabitats it was as low as 6.8. There was a seasonal temperature variation of 20°C (9°C - 29°C). Freezing or near freezing temperatures were also occasionally recorded during 1961 and 1963. A maximum temperature of 32°C was measured in certain microhabitats.

Breeding occurred throughout the year, but maximum reproduction and large colonies were observed from April through July or early August.

Associate snails were *Helisoma trivolvis lentum* Say, *Helisoma costaricense* Preston, *Lymnaea (Pseudosuccinea) columella* Say, *Physa anatina* Lea, *Littoridina monroensis* Frauenfeld, *Lyrodes coronatus* Pfeiffer, *Gyraulus* sp., *Amnicola limosa* Say, *A. integra* Say, *A. binneyana* Hannibal, *Viviparus georgianus* Lea, *Campeloma floridense* Call.

The most common aquatic plants in the snail habitats are water lentils, *Lemna* spp.; coontail, *Ceratophyllum demersum;* pondweeds, *Potamogeton* spp.; water lilies, *Nymphaea odorata;* and some unidentified species of water mosses.

Some commensals, parasites and predators were observed in and on the tropicorbid snails in Louisiana. Among the important and most common of these were the oligochaete *Chaetogaster limnae*, and some larval trematodes. Infection with strigeids was encountered in 3 colonies, and echinostome metacercarial infection in another 3 colonies. By feeding experiments to pigeons, this echinostome was proved to be *Echinoparyphium recurvatum*. In another colony, at Wilswood pond, the snails were shedding cercariae of the psilostome *Psilostomum ondatrae*, as determined later by infecting fish (second intermediate hosts) as well as mammalian hosts.

Florida and Texas: In Florida, Dr. H. Leigh found the snails to inhabit drainage ditches in and near Miami. In Texas they were collected by Dr. E. G. Berry from lakes, roadside ditches, a fish hatchery and from certain creeks, apparently in the prairie area in the southern and central part of the State.

At present there is no definite explanation for the absence of the snails

from the State of Mississippi. Although mixed bottomland hardwoods, as they occur in Louisiana, are found in the southern portion of Mississippi, the prevailing trees are longleaf and shortleaf-loblolly pines, which are continuous with similar forest types in the central part of the State. The presence of such habitats might explain the absence of the snails because in Louisiana also the snails were not located in pine areas.

TROPICORBIDS COLLECTED FROM OTHER PARTS OF THE AREA

The collection sites are indicated at appropriate places in the text, together with the materials examined.

The habitats from which the snails were collected by the writer included a swamp near Central Farm, British Honduras; Gatun Lake and a roadside ditch in Gamboa, Panama Canal Zone; and also flood plains and large swampy areas between Villahermosa and the Gulf of Mexico, in the State of Tabasco, Mexico.

MORPHOLOGY

A. CONCHOLOGICAL AND ANATOMICAL EXAMINATIONS

Specimens from the areas and localities listed below were examined and identified by the author as follows. The numbers quoted include shells and animals.

1. U.S.A.:
   Snails from several localities in Louisiana (collected by the author); 1 locality in Florida (collected by Dr. Henry Leigh); 2 localities in Texas (provided by the late Dr. Asa C. Chandler). All *Biomphalaria obstructa* (Morelet, 1849); 1145 specimens; Figs. 2a, b, 9-11, 21a.
2. Puerto Rico:
   Biomphalaria obstructa (Morelet 1849), 30 specimens.
   B. albicans (Pfeiffer 1839), 15 specimens.
   B. riisei (Clessin 1884), 12 specimens; Fig. 20.
   Material collected by Dr. Charles S. Richards.
3. Dominican Republic:
   Biomphalaria havanensis (Pfeiffer 1839) from Rio Nigua, near San Cristobal, 45 specimens; Figs. 6, 14. Collected by Dr. Frank Etges.
4. Jamaica:
   Biomphalaria pallida (C. B. Adams 1846) from near Kingston, 18 specimens; Figs. 5, 15. Collected by Dr. Harold Harry.
5. Venezuela:
   Biomphalaria straminea (Dunker 1848), 45 specimens, from Guatire and Guaraymito (albino) in the States of Miranda and Aragua respectively; Fig. 16.
   B. schrammi (Crosse 1864) from Guacara, Carabobo State, 30 specimens; Fig. 17.
   Material collected by Mr. P. Chroschiesowski.
6. British Honduras:
   Biomphalaria albicans (Pfeiffer 1839), from near Central Farm, 45 specimens; Figs. 8, 18a,b. Collected by the author.
7. Costa Rica:
   Biomphalaria albicans (Pfeiffer 1839) from near Turrialba, 14 specimens; Fig. 19. Collected by Mr. Gary Pace.
8. Panama:
   Biomphalaria fieldii (Tryon 1863) from Canal Zone at Gatun Lake and Gamboa, 45 specimens; Figs. 7, 12. Collected by the author.
9. Brazil:
   Biomphalaria peregrina (d'Orbigny 1835) from Cel Felicia Lima, in the State of Minas Gerais, 45 specimens; Figs. 13, 21b. Collected by the author.

Key to Abbreviations

al.g., albumen gland; ca., carrefour; f.g.o., female genital opening; h.d., hermaphrodite duct; m.g.o., male genital opening; mu.g., muciparous gland; ovd., oviduct; ovt., ovotestis; pre., preputium; pro.g., prostate gland; sp.d., sperm duct; s.r.d., seminal receptacle duct; s.r.s., seminal receptacle sac; s.v., seminal vesicle; ut., uterus; va., vagina; va.p., vaginal pouch; v.d., vas deferens; ve., verge; v.s., vergic sac.

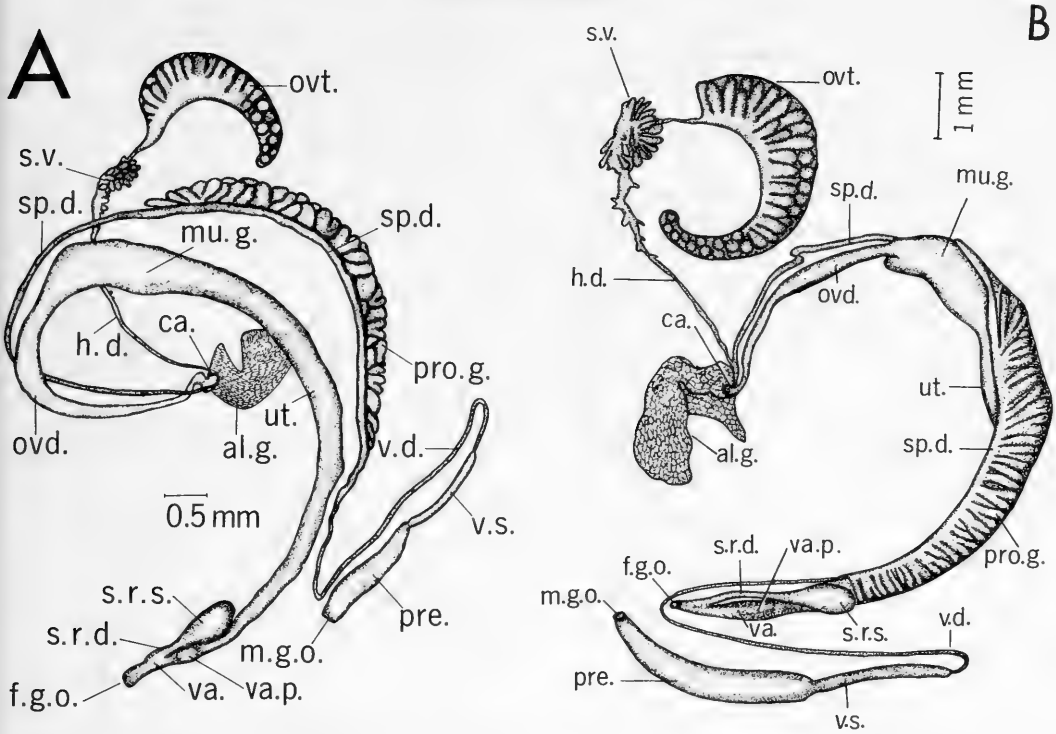

FIG. 2. Genitalia of *Biomphalaria obstructa*. **a.** From Lake on Louisiana State University Campus, Baton Rouge, U.S.A. **b.** From the vicinity of Brownsville, Texas, U.S.A. Note apparent similarities in length ratio of female tract to oviduct, penial complex, hermaphrodite duct, spermathecal duct and sac, and to prostate.

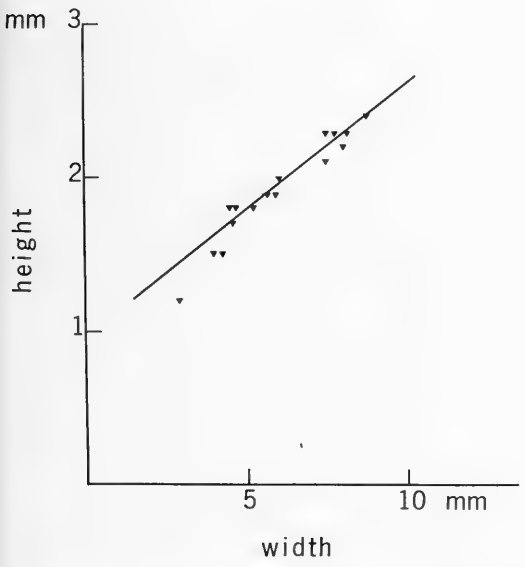

FIG. 3. *Biomphalaria obstructa* from Lake on Louisiana State University campus, Baton Rouge. Diagonal line represents linear regression of shell width on shell height.

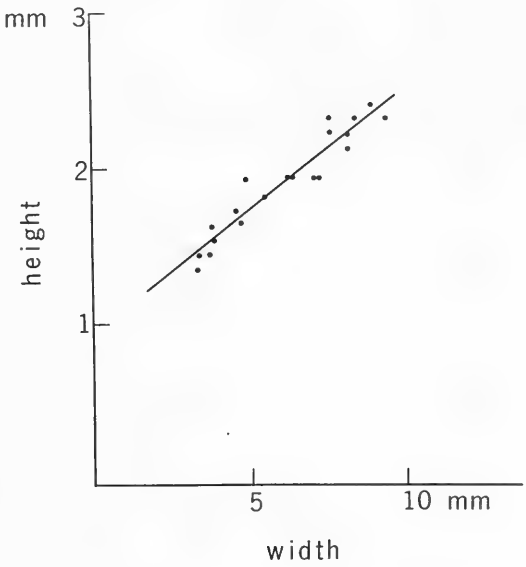

FIG. 4. *Biomphalaria obstructa* from Dade County, Florida, U.S.A. Diagonal line represents linear regression of shell width on shell height.

1. Basis for Grouping

The Shell

The tropicorbid snails are small planorbids whose shells are closely similar. The diameter in these species reaches 13 mm and the height 4 mm; the adult shell has up to 5 whorls increasing slowly or rapidly in diameter; the sutures are distinct; the upper (right or umbilical) side the lower (left, apical or spire)[4] side vary from flattened to broadly concave; the whorls are round; the aperture is ovate or heart-shaped, deflected or not deflected, and may or may not have lamellae at one stage in the growth of the snail.

These features of the shell have proved to be diagnostic and have been used in this study in first grouping the tropicorbids examined as follows:

Biomphalaria obstructa, *B. havanensis*, *B. pallida* and *B. fieldii*
Biomphalaria riisei
Biomphalaria straminea
Biomphalaria albicans
Biomphalaria schrammi
Biomphalaria peregrina

This preliminary grouping on the basis of the shell, which revealed closer similarities between the first 4 species, was subsequently modified after studying the anatomy of the genital tract (see under "Grouping," p 22 ff.).

The Animal

The radula showed similarity among the species examined as well as considerable intraspecific variation. In some specimens of *Biomphalaria peregrina* the lateral teeth had a blunt mesocone (Fig. 21b), whereas in *B. havanensis*, *B. pallida*, *B. fieldii*, *B. obstructa*, *B. riisei*, *B. albicans* and *B. schrammi*, the mesocones on the lateral teeth are consistently more pointed and sharper. The radula of *B. straminea* was not examined.

None of the tropicorbids grouped under the 9 species named above had the longitudinal pigmented ridge on the ventral surface of the kidney characteristic for *Biomphalaria glabrata*.

As regards the genital tract, the ovotestis was predominantly unbranched.

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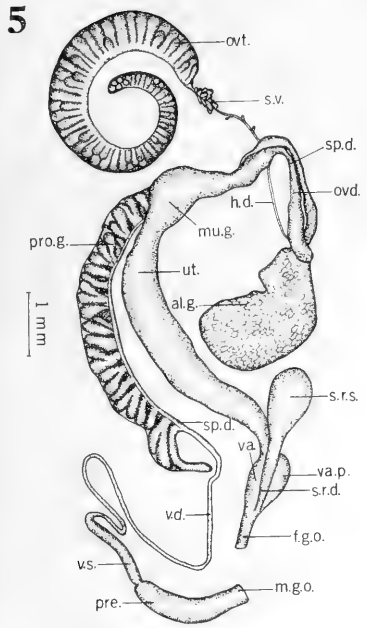
[4]Because some confusion might arise from the varying terminologies used by different authors with regard to the morphology of the planorbid shell, a definition of terms and orientation will be given in the following. "Right" and "left" are the shell surfaces so oriented, when the discoidal shell is held in its natural position on the living animal with the plane of coiling vertical, and hence also when the shell is rotated a little in that same plane and viewed with the aperture at the bottom so that it faces the observer, as shown in the apertural views in Figs. 9 - 20. The shell, however, is not borne vertically in life, but, in the planorbids of this group, is tilted more or less to the left. The left is therefore also the "lower" side, the opposite, right side being the "upper" side. It is generally understood that the left side, which in *Biomphalaria* usually shows a more or less pronounced concavity, is the side which in other sinistrally organized snails would show the elevated spire, and that it thus corresponds to the "apical" side. The opposite, upper, right side is frequently termed the "umbilical," i. e., the side which, in an umbilicate spiral shell, would show an umbilcus at the base of the columella. However, the pronounced navel-like depression on the underside of African *Biomphalaria* has been called "umbilicus" by Mandahl-Barth and other authors, their umbilical side thus corresponding to the apical or "spire-pit" side of other authors. As Mandahl-Barth has pointed out in his monograph on African *Biomphalaria* (1958: 13): "The umbilicus, that is the concavity on the underside of the shell . . . corresponds not with the umbilicus of other shells, but with the spire." ED.



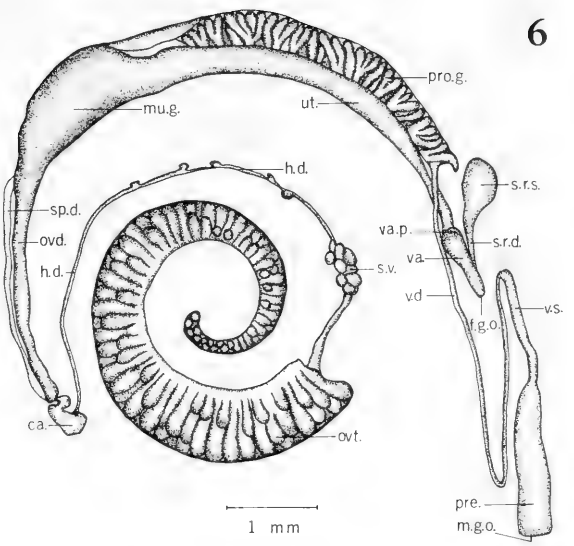
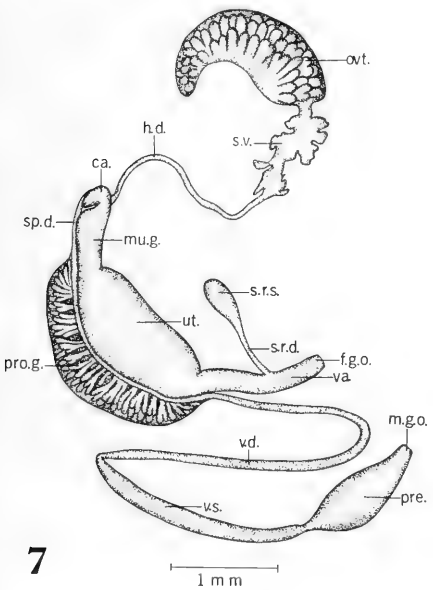


FIG. 5. Genitalia of *Biomphalaria pallida* from Kingston, Jamaica. Note large, pear-shaped vaginal pouch; vergic sac is shorter than preputium, prostate long as compared to female tract.

FIG. 6. Genitalia of *Biomphalaria havanensis* from Rio Nigua, near San Cristobal, the Dominican Republic. Note vaginal pouch, long hermaphrodite duct. Albumen gland removed.



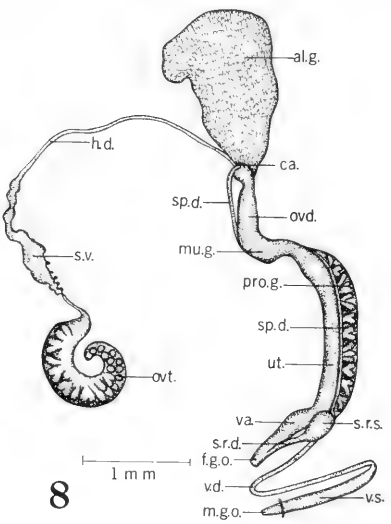


FIG. 7. Genitalia of *Biomphalaria fieldii,* from Gatun, Panama. Note absence of vaginal pouch, the long prostate and penial complex as compared to length of female tract, and that the vergic sac is much longer than the preputium. Albumen gland removed.

FIG. 8. Genitalia of *Biomphalaria albicans* from swamps near Central Farm, British Honduras. Note absence of vaginal pouch, very long hermaphrodite duct, length of prostate (almost half the length of genital tract), the very short preputium as compared to vergic sac. The latter 2 structures are almost equal in diameter and some of the penial muscles are shown at the boundary between them.

The genitalia otherwise provided the following characteristics of some interspecific diagnostic value: (1) Presence or absence of a vaginal pouch, of vaginal corrugations or foldings. (2) Length of the prostate gland (i.e. area of the genital tract occupied by the gland). (3) Length ratios of female tract (from the point of bifurcation of the hermaphrodite duct to the female genital opening) to the following organs: oviduct, penial complex, hermaphrodite duct, spermathecal duct and sac, and prostate. (4) Length ratio of preputium to vergic sac.

Table 1 summarizes the diagnostic anatomical characteristics of the species examined from the Gulf of Mexico mainland and the Caribbean areas, comparing them to *Biomphalaria peregrina* from South America (Brazil). Such a comparison seems necessary because of the close relationship of *B. peregrina* to the northern species under consideration.

2. Description of the Species examined

To eliminate features due to growth variation, only adult specimens have been considered. When sufficient material was available, up to 30 shells of each population were examined and 15 animals dissected. The numbers were smaller in 3 of the 9 species: *Biomphalaria riisei*, *B. schrammi* and *B. pallida* (see under specimens in materials listed above, and numbers dissected in Table 1 under each species.

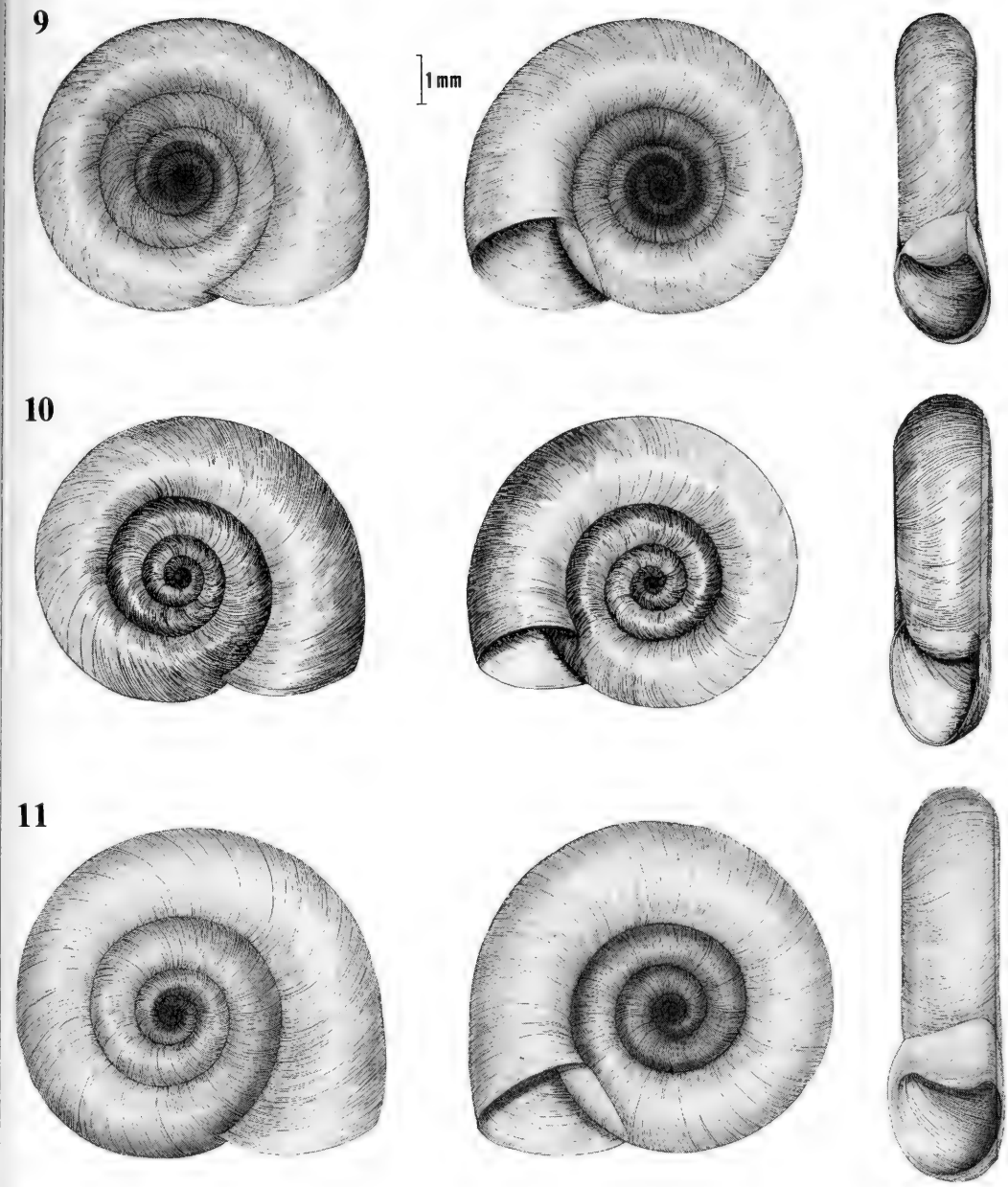
Biomphalaria obstructa
Figs. 2a, b, 9, 10, 11

Louisiana Material (24 localities)

The Shell. A total of 720 shells from the various populations were examined. The diameter of the shell in the Louisiana specimens does not exceed 9 mm nor the height 2.5 mm. The shell has 5 whorls. It is biconcave; on the left (lower, apical or spire) side, the concavity is shallow but in 2 lots from Baton Rouge (Capitol lake and lake across from Auditorium on Louisiana State University (L.S.U.) campus, Fig. 10) the concavity is deep, forming a sunken spire. On the right (upper, or umbilical) side it is shallow, with a central notch. The periphery of the whorls is round and the sutures are distinct. The aperture is heart-shaped with distinct lamellae in some young specimens. Figure 3 shows the relationship between the shell width and shell height of one colony (L.S.U. campus).

The Animal. A total of 360 animals from Louisiana were examined. The exterior of the animal is pale grey. The mantle is studded with distinct rounded or elongated black or grey spots which show considerable variation within each colony examined. Generally, when the laboratory raised progenies were compared to field collected specimens, pigmentation of the mantle was noticeably reduced. In the pallial cavity, there is no renal ridge on the ventral surface of the kidney. The dorsal mantle ridge and rectal ridge are both present and quite distinct. The rectal ridge is transversely folded several times, but also shows straight unfolded portions.

The mouth opening is guarded by a jaw which consists of a strong brown dorsal portion, and a slender portion on each side. The teeth of a radular half row on either side of the central are: 7 or 8 laterals, 1 - 2 intermediates, and, from the 10th tooth on, 9 - 11 marginals. The mesocone on the lateral teeth is usually sharp and pointed (Fig. 21a). The salivary glands join distad of the buccal mass. In the reproductive tract (Fig. 2a), the prostate has 18 - 27 branched tubules. It was 2.7 - 4.3 mm long in specimens 5.5 - 8 mm in diameter (7.5 mm average). There is no separate prostate duct, and the prostate diverticula were never found to reach 50 in number, as described by Baker (1945) for specimens from New Orleans identified by him as *T. havanensis*.



FIGS. 9–11. Shells of *Biomphalaria obstructa* in right (upper, umbilical), left (lower, spire) and apertural view (compare with footnote[4], p 12 and note that the drawing on the left shows the right side of the shell in relation to the living animal). FIG. 9. From Dade County, near Miami, Florida, U.S.A. FIG. 10. From Lake on Louisiana State University, Baton Rouge, Louisiana, U.S.A. FIG. 11. From Bonnet Carré Spillway near Norco, Louisiana, U.S.A.

In these specimens, the sperm duct, from the bifurcation of the hermaphrodite duct to the first prostate tubule, was 2.7 - 3.6 mm long; the spermathecal duct and sac 1.- 1.5 mm. The length ratio of the whole female tract to the oviduct is 1:0.32, and to the penial complex it is 1:0.41. The proximal end of the vagina adhering to the spermatheca usually shows on its right side (i.e., that adhering to the left side of the spermatheca) a rudimentary pouch outlined by pigment: in some specimens there is only a thickening instead, also outlined by pigment.. The vergic sac is thinner than, and slightly shorter or equal to, the preputium (the former being 1.2 - 1.6 mm long and the latter 1.26 - 1.85 mm).

Eggs. In the laboratory egg masses were laid all-year-round on the vegetation, on the glass wall of the aquaria, or on the enamel coat of the pans. The eggs of *Biomphalaria obstructa* averaged 0.55 mm in diameter. At the onset of egg laying, the snails varied in diameter from 4 - 6.5 mm. Some young snails were reared in isolation from the day they hatched and observed until they matured and laid eggs. This period ranged between 38 and 52 days at 22 - 25°C.

Florida and Texas Material

In their shell characteristics the specimens of *Biomphalaria obstructa* from Texas near Brownsville (30 specimens examined) and Florida (35 specimens) did not differ, in the main, from those obtained in Louisiana. Figs. 3 and 4 demonstrate that for Florida and Louisiana specimens, the linear regression of shell width on shell height is similar. In the Florida population, however, the left (lower) side is consistently flatter than the corresponding side of the Louisiana and Texas specimens, and there is a tendency for each whorl not to overlap the preceding one. The soft anatomy of specimens from Florida and Texas does not differ from that of the Louisiana material. In 15 animals from the Texas specimens with larger shells i.e. 10 - 11 mm in diameter, the genitalia (Fig. 2b) showed the following average measurements[5]: prostate 4.5 mm; sperm duct to first prostate tubule 4.2 mm; spermathecal duct and sac 2.2 mm; length ratio of female tract to oviduct 1:0.30, and length ratio of female tract to penial complex 1:0.38; length of vergic sac 2.3 mm, and of preputium 2.8 mm.

Puerto Rican Material

Biomphalaria obstructa from Puerto Rico (15 shells and 15 animals examined) agreed in the main with the same species from Louisiana and Texas.

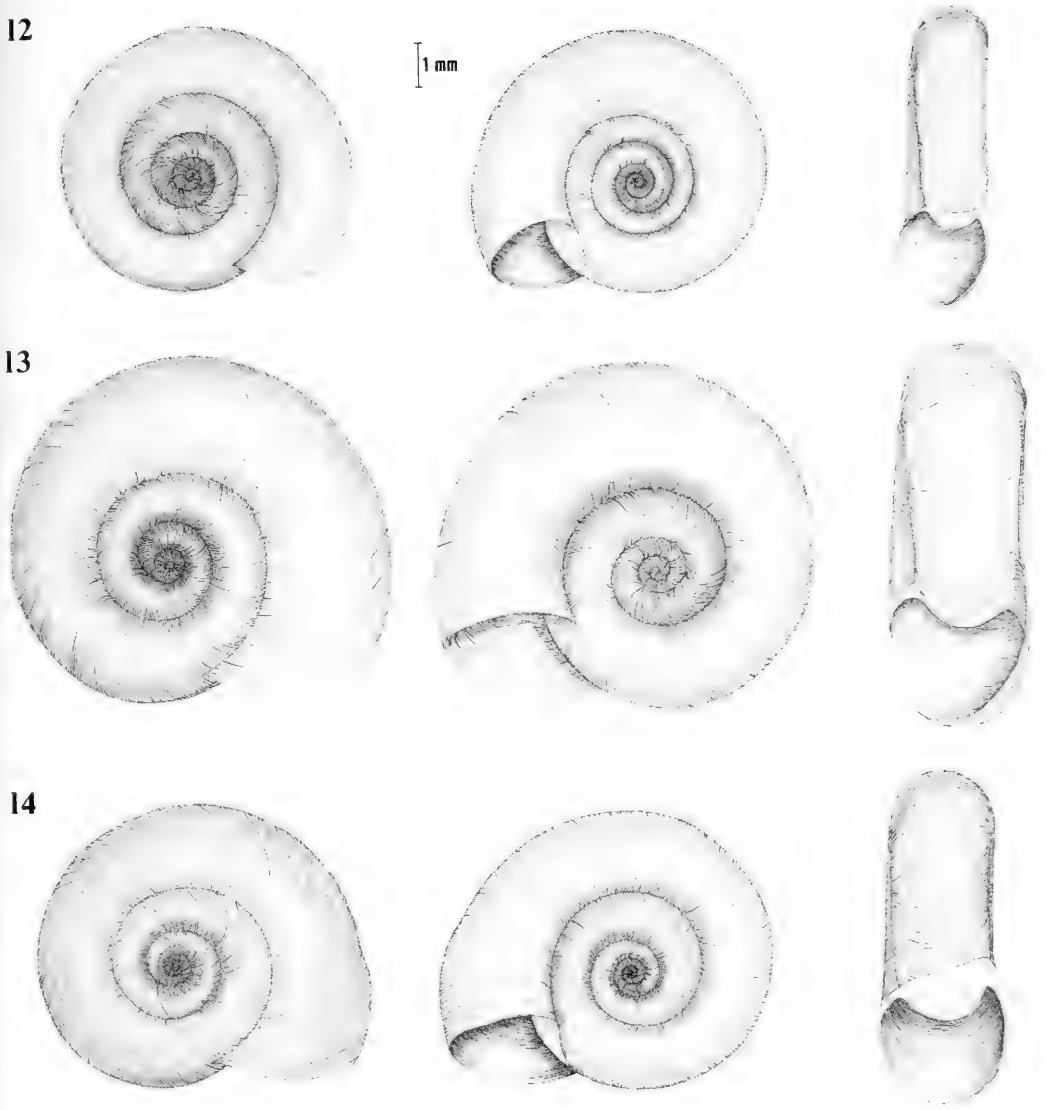
Biomphalaria pallida
Figs. 5, 15

Jamaican Material. Vaginal pouch present: large, pearshaped. In 8 snails with shells averging 7.5 mm in diameter and 1.5 mm in height: prostate gland 5.3 mm long, vergic sac (1.4 mm) slightly shorter than preputium (1.8 mm); length ratio of whole female tract to penial complex 1:0.38, to oviduct 1:0.31, length of spermathecal duct and sac 2.4 mm; seminal vesicles finger-like protrusions (Fig. 5).

Biomphalaria havanensis
Figs. 6, 14

Material from the Dominican Republic. In the genital tract, vaginal pouch poorly to well developed. In 15 snails with shells averaging 7.3 mm in diameter and 1.8 mm in height, prostate gland 3.3 mm long, vergic sac (1.3 mm) slightly shorter than preputium (1.7 mm), length ratio of whole female tract to penial complex as well as to oviduct 1:0.33 mm; length of spermathecal duct and sac 1.4 mm, seminal vesicles

[5]Unless otherwise stated, the measurements given for other species are also averages.



FIGS. 12-14. Shells of *Biomphalaria* in right (umbilical), left (spire), and apertural view. FIG. 12. *B. fieldii* from Gatun, Panama. FIG. 13. *B. peregrina* from Cel Felicia Lima, near Ruiz de Fora, Brazil. FIG. 14. *B. havanensis* from the Dominican Republic.

finger-like (Fig. 6). This snail from the Dominican Republic has been identified by local workers as *B. riisei*. However, its shell differs from that of *B. riisei*, it has a less developed vaginal pouch, a longer oviduct and the ratio of preputium to vergic sac is different in the 2 snails.

Biomphalaria fieldii
Figs. 7, 12

The shell of this species reaches up to 10 mm in diameter and 2.5 mm in height. The whorls are round and smooth; in some specimens the early whorls may show a slight carination. Left side with a broad concavity, the right with a dimple-like narrow depression.

In 15 specimens examined from the type locality in Gatun, Panama, and from Gamboa, averaging 6 mm in shell diameter: no vaginal pouch, vergic sac (2.25 mm) much longer than preputium (1.25 mm) resulting in the unusual ratio of 1:1.8 (Table 1); spermathecal duct and sac 1.1 mm and the prostate 2.4 mm. Length ratio of female tract to oviduct 1:0.44, and to penial complex 1:0.90.

Biomphalaria riisei
Fig. 20

The material examined was from Puerto Rico. The diameter of the shell reached 13 mm, the height 3 mm at the beginning of the last whorl and 4 mm at the aperture. In many specimens carination of the whorls is quite pronounced on the lower (left) side; the whorls are slowly increasing; the right side is flat with a pronounced broad central concavity, while the left side has a narrow but pronounced depression. In general the aperture is not deflected. Anatomically, my material conformed in the main with that described by Richards (1964), who, however, examined more material, both field-collected and laboratory-reared, than was available to me. In my study of 6 specimens with a shell diameter of 11 mm and shell height of 3 mm, the prostate was 2.8 mm long; the vergic sac 1.5 mm, the preputium 1.4 mm, the spermathecal duct and sac 1 mm, the length ratio of the female tract to oviduct 1:0.27, and to penial complex 1:0.44.

Biomphalaria straminea
Fig. 16

The shell of this species is known to reach 14 mm in diameter and 3 mm in height. The Venezuelan specimens here examined measured 9 mm in diameter maximally, and 2.9 mm in height.

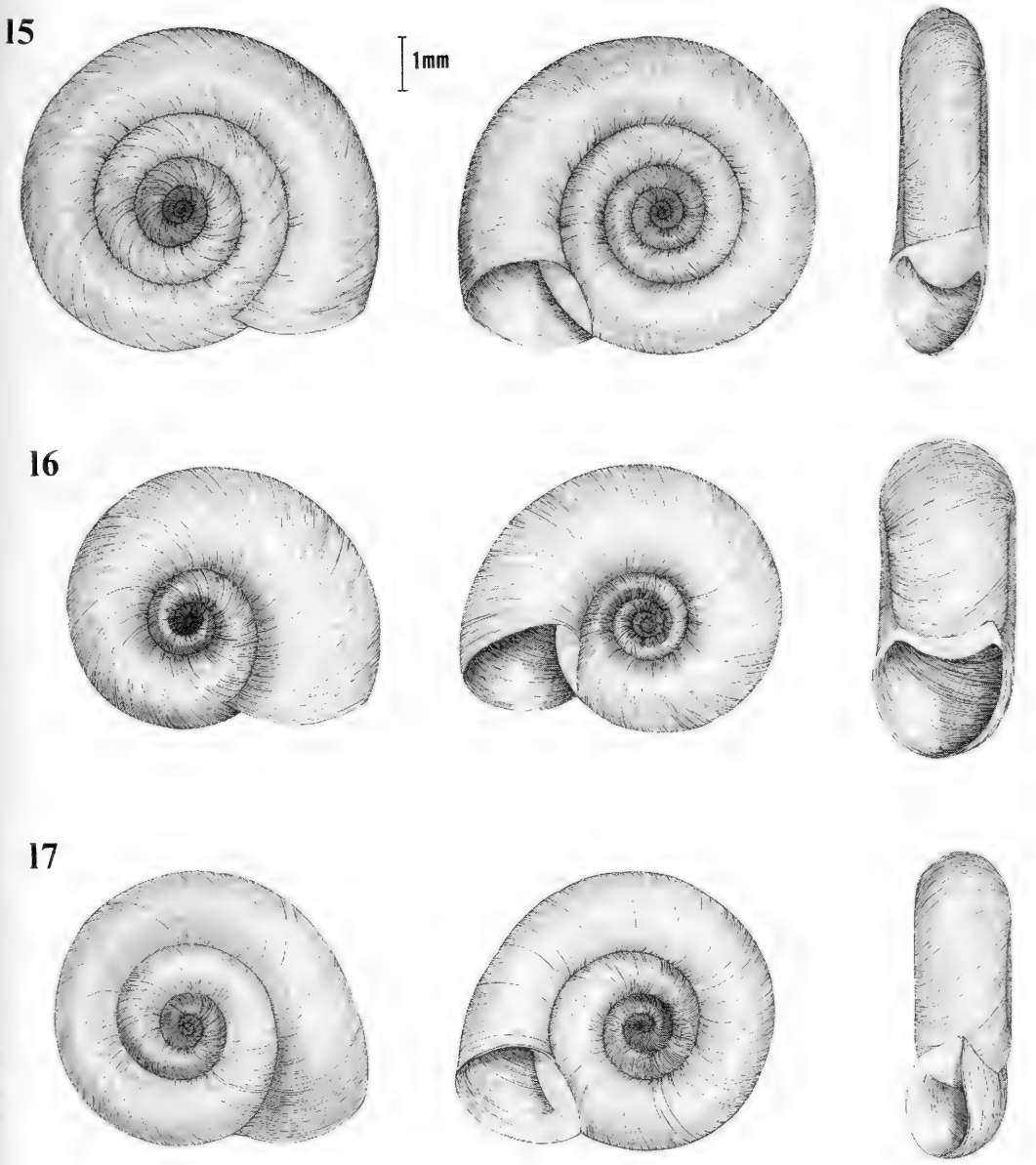
The whorls increase slowly, except for the last whorl, which increases rapidly and thus forms a wide aperture. The left side shows a deep concavity (spire-pit), and the right side is broadly concave with central depression.

The snails from Venezuela had no vaginal pouch, but corrugations in the form of transverse folds are found on the side of the vagina adhering to the spermatheca. In 15 specimens with shells averaging 8 mm in diameter and 2.7 mm in height, the prostate gland was 2.5 mm long; the vergic sac was slightly shorter (1.2 mm) than the preputium (1.3 mm); the length ratio of the female tract to the penial complex was 1:0.50 and that to the oviduct 1:0.32; the length of the spermathecal duct and sac was 1 mm; the seminal vesicles were finger-like.

Biomphalaria albicans
Figs. 8, 18a,b, 19

Material from swamps near Central Farm in British Honduras. The species is small with a shell up to 7 mm in diameter and 2.5 mm in height; 3 - 4 rapidly increasing whorls slightly carinated on both sides, sometimes rounded on the right side. On that side there is a deep central depression; on the left, the concavity is less pronounced. Aperture usually not deflected; the apertural region is provided with a number of lamellae (up to 6).

Laboratory cultures were established.



FIGS. 15-17. Shells of *Biomphalaria* in right (umbilical), left (spire) and apertural view. FIG. 15. *B. pallida* from Jamaica, near Kingston. FIG. 16. *B. straminea* from Guatire, State of Miranda, Venezuela. FIG. 17. *B. schrammi*, from Guacara, State of Carabobo, Venezuela.

The progeny showed intrapopulation variation as to the carination of the whorls on both sides of the shell and the number of apertural lamellae which varied from 3 - 6.

There was no vaginal pouch. In 15 snails, with shells averaging 5 mm in diameter and 2.0 mm in height, the vergic sac (1.0 mm) was much longer than the preputium (0.2 mm); the spermathecal duct and sac measured 0.7 mm, the prostate 1.9 mm. Length ratio of female tract to oviduct was 1:0.34, and to penial complex 1:0.33; length ratio of preputium to vergic sac 1:5.0.

Biomphalaria schrammi
Fig. 17

The shells of this species, especially of the lamellate form, show similarity with those of *B. albicans*, except that they are slightly lower, and usually calloused at the aperture on the parietal wall.

In 10 snails from Venezuela, averaging 5 mm in shell diameter and 1.8 mm in height, the prostate gland was 3.8 mm long; the vergic sac much longer (5 mm) than the preputium (0.8 mm); the length ratio of the female tract to the penial complex was 1:0.40, and that to the oviduct 1:0.29; the spermathecal duct and sac are long (3.8 mm).

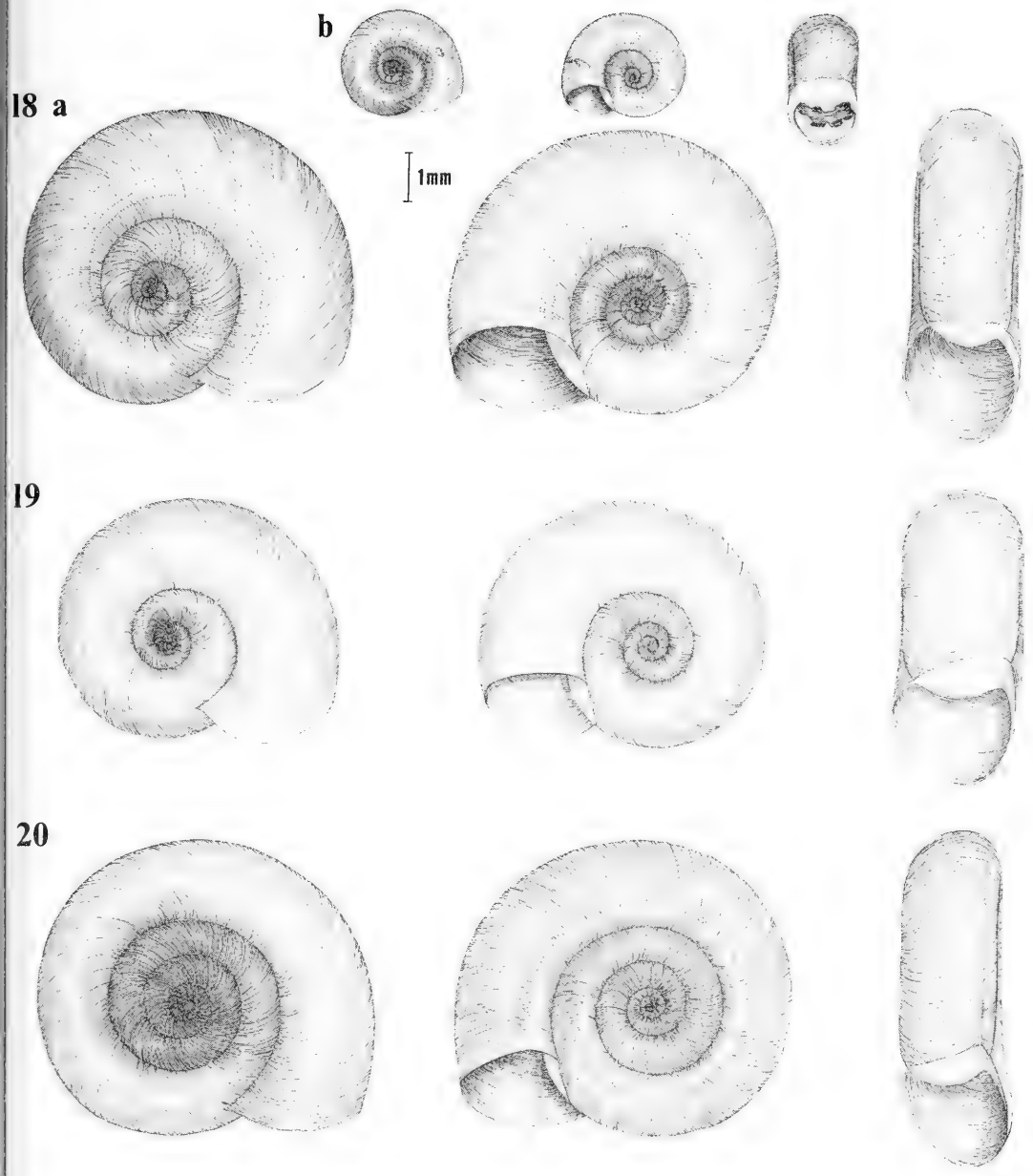
Biomphalaria peregrina
Figs. 13, 21b

A description of this South American species, based on the examination of 15 animals and 30 shells that were collected by the writer from Cel Felicia Lima, near Ruiz de Fora, Brazil, is included in this study for comparison with the species studied from the Caribbean and the Gulf of Mexico area, even though this species does not occur within the geographical area discussed. Shells are up to 12 mm in diameter and 4 mm in height at the aperture, and 3 mm at the beginning of the last whorl. The slowly increasing whorls are rounded on the periphery, but show carination on the left side. Sutures are deep. The right side has a sunken "umbilical" concavity, while the left side shows a wide and deep apical depression, which differentiates this species from the group comprising the otherwise rather similar *Biomphalaria obstructa*, *B. havanensis*, *B. pallida* and *B. fieldii*. The aperture is heart-shaped and flaring out towards the left side.

In 15 specimens with a shell diameter averaging 10 mm, a height of 2.9 mm at the beginning of the outer whorl, and 3.6 mm at the aperture, the measurements of organs in the genital tract were as follows: length of prostate gland 4.5 mm; vergic sac (2.7 mm) almost equal to preputium (2.6 mm); length ratio of female tract to penial complex 1:0.50, and to oviduct 1:0.30; length of spermathecal duct and sac 1.7 mm.

B. MATERIAL EXAMINED CONCHOLOGICALLY

In addition to the material collected privately, the shells of some 30 "species" or forms of tropicorbids, from the Gulf of Mexico, northern South America and Caribbean area, that were present in the museum collections of the Mollusk Division, University of Michigan, the Mollusk Division of the U.S. National Museum in Washington, D.C., and the Instituto Nacional de Endemias Rurais at Belo Horizonte, Brazil, were also studied. This material comprised 85 lots of which about 900 snails were examined. Shells of *Biomphalaria peregrina* from Brazil were also viewed. The nominal species examined were: *B. albicans*, *B. cannarum*, *B. centimetralis*, *B. decipiens*, *B. declivis*, *B. dentiens*, *B. dentiens edentula*, *B. dentifera*, *B. donbilli*, *B. fieldii*, *B. geoscopa*, *B. havanensis*, *B. isthmica*, *B. janeirensis*, *B. kühniana*, *B. liebmanni*, *B. maya*, *B. meridensis*, *B. obstructa*, *B. obstructa donbilli*, *B. obvoluta*, *B. orbicula*, *B. orbicula dunkeri*, *B. pallida*, *B. peregrina*, *B. planulata*, *B. retusa*, *B. riisei*, *B. schrammi*, *B. shimeki*, *B. straminea*, *B. terveriana*.



FIGS. 18-20. Shells of *Biomphalaria* in right (umbilical), left (spire), and apertural view. FIG. 18. **a.** *B. albicans* from swamp near Central Farm, British Honduras. **b.** *B. albicans*, young lamellate forms from same locality. FIG. 19. *B. albicans* from Costa Rica. FIG. 20. *B. riisei* from Puerto Rico.

For the names of all species reported from the area concerned the reader is referred to Harry (1962).

GROUPING OF FORMS EXAMINED

As a result of this study and taking into consideration the findings on the material described above and, in part, of the synonymies already proposed by previous workers (Paraense, 1963; Paraense & Deslandes, 1957a,b), it is proposed to divide the tropicorbids of the area in question into 5 groups comprising 8 valid species as outlined below. The first 3 groups (6 species) are considered to be part of a larger complex, including *Biomphalaria peregrina*, a South American species.

I. The *Biomphalaria obstructa* group.[6]

This group comprises 4 closely related species probably having the same recent common ancestor: *Biomphalaria obstructa*, *B. havanensis*, *B. pallida* and *B. fieldii*. They are forms in which the shell is flat on both sides and compressed, especially when compared with the shell of members of the other groups. Its height does not exceed 2.5 mm, nor the diameter 11 mm. The shell has slowly increasing whorls, rarely exhibiting carination; the left side has a shallow but broad concavity, while the right may have a narrow central notch. The geographical distribution of this group comprises the southern United States, Mexico, Central America and some islands of the Caribbean. Each of these species and its synonymies is dealt with separately below.

(1) *Biomphalaria obstructa* (Morelet 1849); type locality: Carmen Island, Campeche, Mexico. The following 4 species and varieties should be placed in its synonymy: *B. obstructa donbilli* (Tristram 1861), *B. meridensis* (Preston 1907), type locality: Merida, Yucatan; *B. orbicula* (Morelet 1849), type locality: Carmen Island, Campeche; and *B. orbicula dunkeri* (Baker 1945).

(2) *Biomphalaria havanensis* (Pfeiffer 1839); type locality: Havana, Cuba. Paraense & Deslandes (1957a,b), after examination of topotypical material, have suggested the synonymy, with *B. havanensis*, of *B. terveriana* (d'Orbigny 1841), type locality: Havana, Cuba; *B. liebmanni* (Dunker 1950), type locality: Vera Cruz, Mexico; *B. maya* (Morelet 1849) type locality: Campeche, Mexico; and *B. retusa* (Morelet 1841), type locality: Carmen Island. To these synonyms can be further added *B. obvoluta* (Clessin, 1885), type locality: Havana, Cuba.

(3) *Biomphalaria pallida* (C.B. Adams 1846); type locality: Kingston, Jamaica. *B. decipiens* (C. B. Adams 1849); type locality Jamaica, should be placed in synonymy.

(4) *Biomphalaria fieldii* (Tryon 1863), type locality: Gatun, Panama. Synonym: *B. isthmica* (Pilsbry 1920), type locality: Panama city, Panama.

II. *Biomphalaria riisei* "Dunker" (Clessin 1883), type locality: "Jamaica, Puerto Rico."

Shell is high (see p 18). Carination of the whorls is pronounced on the left side, though non-carinated forms are encountered. The larger of these are similar to young, or small size, *Biomphalaria glabrata* in Puerto Rico. The geographical distribution as far as is known, is Puerto Rico, the Dominican Republic and Jamaica.

III. *Biomphalaria straminea* (Dunker, 1848); type locality: "South America."

The shell has slowly increasing whorls but a wide aperture and a deep apical concavity on the left side (see p 19). The geographical distribution covers northern South America, reaching into the area surrounding the Caribbean. Paraense (1963) regarded *B. centimetralis* (Lutz 1918) from northeastern Brazil and *B. kuhniana* "Dunker" (Clessin 1883) (type locality: Surinam or Dutch Guiana) as synonyms of *B. straminea*. The Venezuelan specimens

[6]Specimens collected by the writer in the State of Tabasco, Mexico, from swamps 10 miles northeast of Villahermosa, and others from swamps between Villahermosa and the Gulf of Mexico are conchologically diagnosed as *Biomphalaria obstructa*.

examined in this study agree well in shell characteristics with specimens of *B. centimetralis* from Pernambuco (northeastern Brazil) and also with *B. kuhniana* from Georgetown, British Guiana, and *B. kuhniana* from Surinam.

Paraense (1963) also listed *Biomphalaria paparyensis* (Baker 1914) and *B. incerta* (Lutz 1918) as synonyms, but later (1964) considered these South American species synonymous with *B. schrammi*.

IV. *Biomphalaria albicans* (Pfeiffer 1839); type locality: Cuba.

These are small snails up to 7 mm in diameter and considering their small size the shell is high (2.5 mm); whorls rapidly increasing, with slight carination on right and left sides; apertural region is provided with up to 6 lamellae. Geographical distribution mainly in Central America but also on some Caribbean islands.

On the basis of the study of a large number of the shells of the 9 nominal species listed below it is apparent that they should be included into the synonymy of *Biomphalaria albicans: B. declivis* (Tate 1870), type locality: Acoyapa, Nicaragua; *B. donbilli* (Tristram 1861), type locality: Lake of Duenas, Guatemala; *B. geoscopa* (Pilsbry & Brown 1914), type locality Antigua; *B. planulata* (Clessin 1884), type locality: St. Thomas; *B. dentifera* (C. B. Adams 1845), type locality: Jamaica; *B. cannarum* (Morelet 1849); *B. dentiens* (Morelet 1849); *B. dentiens edentula* (Fischer & Crosse 1880); the type locality for the latter 3 forms is British Honduras, Belize; and *B. shimeki* (F. C. Baker 1945), type locality: Ometope, Nicaragua.

V. *Biomphalaria schrammi* (Crosse 1864); type locality Guadeloupe.

Synonym: *B. janeirensis* (Clessin 1884); type locality: Rio de Janeiro, Brazil. This species comprises forms reaching 7 mm in diameter. The shell is calloused at the aperture, and there are lamellate as well as non-lamellate forms. The former are similar to *B. albicans*, though they are slightly lower and not carinate. Anatomically, however, they can be easily differentiated.

The species is represented in the Caribbean, in particular Guadeloupe, and on the mainland in South America.

DISCUSSION

The study has shown that 8 species (including forms which should be regarded as their synonyms) are represented in the Gulf of Mexico and the Caribbean area. These species are *Biomphalaria obstructa, B. havanensis, B. pallida, B. fieldii, B. riisei, B. straminea, B. albicans* and *B. schrammi*.

The above species can be distinguished from one another on the basis of either shell, proportions of various parts of the genitalia, or a combination of both. While the shell is not a reliable tool in differentiating *B. obstructa, B. havanensis, B. pallida* and *B. fieldii*, it can be used in diagnosing the above 4 species as a group from the other 4 species, i.e., from *B. riisei, B. straminea, B. albicans* and *B. schrammi*, which can furthermore be conchologically differentiated among themselves.

The radula showed similarity among the above species, as well as intraspecific variations. *Biomphalaria obstructa, B. havanensis, B. pallida, B. fieldii, B. riisei, B. albicans* and *B. schrammi* had a sharply pointed mesocone on the lateral teeth, by which they may usually be differentiated from *B. peregrina* and its synonyms, a group of forms inhabiting central and southern South America. The latter usually, but not always, possess a blunt and shorter

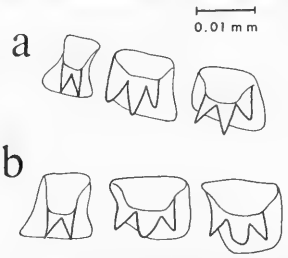


FIG. 21. Central tooth and 2 lateral teeth of 2 species of *Biomphalaria* for comparison of mesocones of laterals. **a.** *B. obstructa* from Bonnet Carré Spillway near Norco, Louisiana, with sharply pointed mesocone. **b.** *B. peregrina* from Cel Felicia Lima, near Ruiz de Fora, Brazil, with blunt mesocone.

mesocone on the lateral teeth.

The genitalia generally provided features of significance in the systematics of these tropicorbid species, but again fewer differences were recognizable among *Biomphalaria obstructa*, *B. havanensis*, *B. pallida* and *B. fieldii* than between this species group and the other 4 species. The vaginal pouch is absent in *B. fieldii*, absent or poorly developed in *B. obstructa*, well developed in *B. havanensis*, and still larger and pear-shaped in *B. pallida*. In 3 members of this group there are almost no differences in the length ratios of the entire female tract to the oviduct (about 1:0.3); only in *B. fieldii* was the ratio 1:0.44. There is, however, a difference in mean ratio of the female tract to the penial complex (mean 1:0.41, range 0.38 - 0.44 in *B. obstructa* from Louisiana; 1:0.33 in *B. havanensis;* 1:0.38 in *B. pallida;* and 1:0.90 in *B. fieldii*). Other differences exist in this group in the proportions of the various organs, as compared to the length of the female tract (see Table 1): *B. havanensis* has a longer hermaphrodite duct (ratio 1:0.60) than *B. obstructa* and *B. pallida* (ratio 1:0.34), but it is not as long as in *B. fieldii* (ratio 1:0.90). *B. pallida* and *B. fieldii* are characterized by relatively longer prostate (ratios 1:0.52 and 1:0.62 resp.), and a longer spermathecal duct and sac (ratios 1:0.25 and 1:0.28 resp.). *B. fieldii* stands out in this group by the length ratio of preputium to vergic sac, which is 1:1.8, as compared to 1:0.7 in *B. obstructa* and *B. pallida*, and 1:0.8 in *B. havanensis*.

Anatomical differences between the other species are: presence of a well-developed vaginal pouch in *Biomphalaria riisei* and its absence in *B. albicans*, *B. schrammi* and *B. straminea;* the latter species possesses corrugations on the vaginal surface. The length ratio of female tract to penial complex is also a differentiating characteristic between them; it is 1:0.5 in *B. straminea*, 1:0.33 in *B. albicans*, and 1:0.40 in *B. schrammi*. These proportions may not suffice in themselves to distinguish the latter 2 species from *B. havanensis*, resp. *B. obstructa*, but *B. schrammi* has a small prostate as compared to the other species, and *B. albicans* has a longer hermaphrodite duct than the other 7 species.

The *Biomphalaria peregrina* group, aside from the inconsistent differentiating characteristic of the mesocone in the lateral teeth of the radula, and its geographic location in the neotropics, proved to have features in their genitalia overlapping with those of the species dealth with above (Table 1). This similarity no doubt indicates close relationship of all these species and it might be justifiable to consider them as one complex. The known geographic range of this complex extends from the central areas of eastern South America northwards into Central America, Mexico, the Southern United States and to some Caribbean islands. As in other complexes of tropical planorbids in the Eastern and Western Hemispheres, it is to be expected that fluctuation, shifting and overlap of features does occur among the members of the complex dealt with here.

It seems necessary to continue with the procurement of more material, topotypic and other, from various parts of the neotropics to further study the several members of this complex. Additional methods, e.g., biochemical and serological might also be used in revising the systematics.

It was one of the objectives in my study to clarify the taxonomic status of the Louisiana "tropicorbids." Berry (1947) identified the tropicorbids as *Tropicorbis obstructus* (=*Biomphalaria obstructa*), with the exception of those collected from the south end of the University Lake, Louisiana State University, Baton Rouge, Louisiana. These he called *T. havanensis* (=*B. havanensis*) only because they resembled specimens so labelled, that had been collected from the same area and are now in the Baker collection in the U.S. National Museum.

TABLE 1. Diagnostic anatomical features in the genital tract of *Biomphalaria* species from the Gulf of Mexico and Caribbean Areas compared to *B. peregrina* from Brazil

Species of *Biomphalaria*	No. of specimens examined	Vaginal pouch	Length ratio[a] of female tract[b] (=1) to					Length ratio preputium: vergic sac
			oviduct	penial complex	hermaphrodite duct	spermathecal duct and sac	prostate	
obstructa Louisiana	360	absent or poorly developed	0.32	0.41	0.34	0.15	0.33	1:0.7
havanensis Dominican Republic	15	poorly to well-developed	0.33	0.33	0.60	0.15	0.36	1:0.8
pallida Jamaica	8	large pear-shaped	0.31	0.38	0.34	0.25	0.52	1:0.7
fieldii Panama	15	absent	0.44	0.90	0.90	0.28	0.62	1:1.8
riisei Puerto Rico	6	well-developed	0.27	0.44	0.51	0.20	0.42	1:1.1
straminea Venezuela	15	absent, but instead corrugation on vaginal surface	0.32	0.50	0.52	0.25	0.40	1:1.5
albicans British Honduras	15	absent	0.34	0.33	1.06	0.18	0.50	1:5.0
schrammi Venezuela	10	absent	0.29	0.40	0.25	0.27	0.27	1:6.2
peregrina Brazil	15	well-developed and large	0.30	0.50	0.61	0.16	0.42	1:1

[a]The length ratios have been calculated from average measurements.

[b]From point of bifurcation of hermaphroditic duct to female opening.

In the same publication, he stated, however that both lots are markedly different from *T. havanensis* (=*B. havanensis*) from the type locality near Havana, Cuba, which he examined. I have examined *"B. havanensis"* shells from the Baton Rouge University campus that were kindly lent to me by Dr. Elmer G. Berry and found them identical with specimens collected in the present study from the same part of Louisiana, and here identified as *B. obstructa*.

It has already been indicated above that *Biomphalaria obstructa* and *B. havanensis* are closely related species, many populations possessing similar shells. However, a few anatomical details do distinguish the two, i.e. *B. havanensis* has a vaginal pouch that is usually well developed, a longer hermaphrodite duct and a smaller ratio of female tract to penial complex.

Morphologically, the southern United States "tropicorbids" belong to one species, *Biomphalaria obstructa*, comprising a few ecotypes and possibly some races.

Whether criteria other than morphological, e.g., biochemical and serological, might reveal distinguishing features among the southern U.S. "tropicorbids" remains to be demonstrated.

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RÉSUMÉ

ETUDES SUR LES "TROBICORBIDES"
(*BIOMPHALARIA:* PLANORBIDAE)
DES CARAÏBES ET DU GOLFE DU MEXIQUE

E. A. Malek

Un grand nombre d' "espèces" de Planorbidés ont été signalées des tropiques du Nouveau Monde. Parmi celles-ci il y a des formes qui sont des hôtes intermédiaires, expérimentaux ou naturels, de *Schistosoma mansoni*, et qui, encore récemment, étaient assignées au genre *Tropicorbis*. Etant donné que leur statut est encore très instable, on a tenté d'examiner un certain nombre de ces formes originaires du Golfe du Mexique et des Caraïbes. Les caractères de la coquille, et, pour quelques populations, la morphologie de l'appareil génital, ont été utilisés afin d'établir l'identité des espèces, d'apprécier la synonymie et de déterminer les regroupements. Dans ce but, des exemplaires de Tropicorbidés ont été collectés par l'auteur ou fournis par des correspondants, à partir d'un grand nombre de localités de l'aire envisagée et les collections de musées ont aussi été étudiées. Pour se conformer à l'Opinion 735 de la Commission Internationale de Nomenclature Zoologique, le nom maintenant employé pour ces mollusques est *Biomphalaria*.

L'étude a montré que les espèces nominalement enregistrées pour cette aire géographique pourraient être maintenant limitées à 8 espèces: *Biomphalaria obstructa, B. havanensis, B. pallida, B. fieldii, B. riisei, B. straminea, B. albicans* et *B. schrammi*. On estime que les 6 premières espèces sont plus étroitement apparentées à l'espèce sud-américaine *B. peregrina*. La coquille ne s'est pas révélée comme un critère sûr pour la différenciation des 4 premières espèces, c'est-à-dire *B. obstructa, B. havanensis, B. pallida* et *B. fieldii;* par contre on a pu l'utiliser pour différencier les 4 autres espèces c.a.d. *B. riisei, B. straminea, B. albicans,* et *B. schrammi,* chacune d'entre elles ayant des caractères conchyliologiques propres. L'appareil génital a fourni les critères suivants ayant valeur de diagnose interspécifique: présence ou absence d'une poche vaginale compte tenu des sillons ou des plis vaginaux; longueur de la prostate; rapport de longueur du tractus femelle (du point de bifurcation du canal hermaphrodite jusqu'à l'orifice femelle) à la longueur des organes suivants: complexe penial; canal hermaphrodite; conduit et sac spermathiques; et prostate; également le rapport de longueur du prépuce à celle du sac de la verge.

RESUMEN

ESTUDIOS SOBRE CARACOLES "TROPICORBIDOS"
(*BIOMPHALARIA:* PLANORBIIDAE)
DE LAS AREAS DEL CARIBE Y DEL GOLFO DE MEXICO,
INCLUYENDO EL DUR DE LOS ESTADOS UNIDOS

E. A. Malek

Un crecido número de "especies" planórbidas se han registrado en las zonas neotrópicas. Entre estas hay formas (comprendiendo huéspedes intermediarios naturales o experimentales de *Schistosoma mansoni*) que hasta hace poco se referian al género *Tropicorbis*. Como el status de esas species es todavía inestable, se intentó investigar algunas de ellas, del Golfo de Mexico y de las Antillas, utilizando

caracteres conchológicos y también, en algunas poblaciones, la morfología genital para control identificativo, evaluando la sinonimia de determinados grupos. El autor colectó y obtuvo de colegas, muestras de varias localidades en esas áreas, y se examinaron también colecciones en museos. En conformidad con la Opinión 735 de la Comissión Internacional de Nomenclatura Zoologica, el nombre usado para estos caracoles es *Biomphalaria*.

El estudio ha demostrado que las especies nominales registradas para esa zona, pueden ahora consolidarse en 8: *Biomphalaria obstructa*, *B. havanensis*, *B. pallida*, *B. fieldii*, *B. rissei*, *B. straminea*, *B. albicans*, *B. schrammi*. Las 6 primeras se consideran, aqui, más relacionadas con la *B. peregrina* de Sud América. La concha probó no ser un elemento seguro en la diferenciación de las 4 primeras especies, *B. obstructa*, *B. havanensis*, *B. pallida* y *B. fieldii*, pero puede ser usada para diferenciar las otras 4, *B. rissei*, *B. straminea*, *B. albicans*, *B. schrammi*, cada una de las cuales es conchologicamente distinta. La genitalia suministró el siguiente criterio de valor para diagnostico interespecifico: presencia o ausencia de saco vaginal; longitud de la glándula prostática; longitudes proporcionales del tracto femenino (desde el punto de bifurcación del ducto hermafrodita al orificio genital femenino), en relacion a los siguientes órganos: complejo penial; ducto y saco de la espermateca; próstata; también la proporción de la longitud del prepucio al saco de la verga.

АБСТРАКТ

ИЗУЧЕНИЕ МОЛЛЮСКОВ ИЗ "TROPICORBID" (BIOMPHALARIA: PLANORBIDAE) ИЗ КАРИБСКОЙ ОБЛАСТИ И ИЗ ОБЛАСТИ МЕКСИКАНСКОГО ЗАЛИВА, ВКЛЮЧАЯ ЮЖНУЮ ЧАСТЬ США.

Е. А. МАЛЕК

Большое количество "видов" планорбид известно из неотропической области. Среди них имеются формы, являющиеся естественными или искусственно (экспериментально) зараженными промежуточными хозяевами *Schistosoma mansoni*, и которые до последнего времени относили к роду *Tropicorbis*. Поскольку их положение в системе было до сих пор весьма неясным, была сделана попытка исследовать ряд этих форм из района Мексиканского залива и Карибского моря. Для этого были использованы - строение раковины и, для некоторых популяций, также строение половой системы для видового идентифицирования, установления синонимии и определения отдельных групп.

Для этих целей материал по тропикорбидам собирался автором или был получен от ряда лиц из различных мест указанных выше областей; были просмотрены также коллекции из музеев.

В соответствии с § 735 Международной Комиссии по зоологической номенклатуре для исследованных моллюсков употребляется название *Biomphalaria*.

Исследование показало, что номинально количество видов в указанной выше области может быть сведено к 8 - *Biomphalaria obstructa*, *B. havanensis*, *B. pallida*, *B. fieldii*, *B. riisei*, *B. straminea*, *B. albicans* *B. schrammi*.

Первые 6 видов оказались более близкими к южно-американскому *B. peregrina*. Было установлено, что строение раковины недостаточно надежное основание для дифференциации первых 4 видов: *B. obstructa*, *B. havanensis*, *B. pallida* и *B. fieldii*, между собой, но что по раковине можно отличать эти 4 вида от остальных четырёх - *B. riisei*, *B. straminea*, *B. albicans* и *B. schrammi*, каждый из которых конхиологически различен.

Гениталии дают критерий для межвидовой диагностики: наличие или отсутствие вагинального кармана и, соответственно - вагинальных морщин или складок; длина железы простаты; соотношение длины женского тракта (от точки бифуркации гермафродитного протока до женского генитального отверстия) и следующих органов: пениального комплекса, гермафродитного протока, протока и мешка сперматеки, простаты; а также отношение длины препуциума к длине мешка пениса.

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A TAXONOMIC STUDY OF SOME SPECIES OF *SEMISULCOSPIRA* IN JAPAN (MESOGASTROPODA: PLEUROCERIDAE)

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ABSTRACT

The purpose of this paper is to establish basic taxonomic concepts for 10 distinct species-group taxa of the freshwater snail genus *Semisulcospira*. Over 30 species and subspecies of this genus had been named from Japan, including the Ryukyu and Ogasawara Islands. In the current study, topotypes of the most prominent of the previously described species were obtained. Two taxa are described here as new, *Semisulcospira habei habei* and *S. habei yamaguchi*.

Topotypes were analyzed in terms of the largest 10% of each population. Data were collected on adult shell morphology, embyro shell characters and intra-brood pouch development of embryo shells. Data were analyzed and presented to permit the reader to understand natural variation in the parameters measured or counted. Data were correlated with the cytological findings of Burch & Davis (1967) and Burch (1968) in order to establish species concepts.

The taxa are relegated to 2 species groups, the *Semisulcospira libertina* group and the *S. niponica* groups. The former is characterized by having a chromosome number of n=18 or 20, adult shells have 7 or more basal cords, and there are numerous (100 or more) young in the female brood pouch (modified pallial oviduct). *S. libertina* and *S. reiniana* are the main species in the complex. *S. kurodai* is placed in the group because the taxon's chromosome number is n=18; the species is, however, considered transitional between the 2 species groups as the adult shells average 5.1 basal cords and there are 35.5 ± 15.4 embryos per female brood pouch.

The *Semisulcospira niponica* species group is characterized by species having low chromosome numbers, n=7 to 14; adult shells have 2 to 6 basal cords and there are few embryos per brood pouch (an average of 25.2 ± 9.8 maximum to 5.2 ± 3.4 minimum, depending on the species). Taxa included in this group are endemic in Lake Biwa and its drainage; they are: *S. niponica, S. decipiens, S. reticulata, S. habei habei, S. habei yamaguchi, S. nakasekoae and S. multigranosa*.

A key to the species is provided to aid in the identification. The utility of traits used in describing the taxa is discussed. Characters of basic importance in defining the species are chromosome numbers, number of basal cords on the adult shell, number of embryos carried by the female, ontogeny of shell sculpture, number of ribs and nodes on the adult shell, embryo size and shape, growth patterns of the embryos in the brood chamber, whorl size attained by the embryo in the female, embryo sculpture and color patterns.

Several traits seem to be particularly subject to inter-population variation. These are adult shell width, spire angle, length of body whorl, embryo microsculpture, apical whorl measurements and adult color patterns. In the *Semisulcospira libertina* species group the presence or absence of ribs and embryo sculpture is subject to such variation. Spire angle is, however, useful in differentiating between several species. The number of whorls and adult shell length are subject to environmental control.

Semisulcospira habei yamaguchi, S. decipiens and *S. multigranosa* are sib-

ling species. Further, *S. multigranosa* is polymorphic by having smooth and ribbed morphs, and 3 color patterns. When data from cytological studies and embryo morphology were correlated, it became evident that these are distinct species. Further distinguishing shell features were then recorded. In Lake Biwa the following species are sympatric: *S. habei yamaguchi, S. decipiens, S. multigranosa, S. reticulata* and *S. niponica*.

The majority of species studied here are endemic to the Lake Biwa area, Shiga Prefecture, Honshu. Several conditions for speciation have been present in Lake Biwa. The lake is ancient (Tertiary) and has had about 1 million years stability; it has an immense lacustrine volume divided into numerous niches. Endemism and sympatry perhaps resulted when the lake level dropped with subsequent elevation of barriers preventing immigration or emigration. The shrinking lake forced the association of numerous organisms within the limits of the current lake basin and the single drainage system of the Setagawa River. Other populations were excluded from the lake or perished. *Semisulcospira kurodai* may be an example of such exclusion. A million years of stability probably allowed further speciation or incipient speciation, e.g. *S. habei habei* and *S. habei yamaguchi*.

CONTENTS

I. INTRODUCTION

The freshwater snail genus *Semisulcospira* is widespread in Japan, Korea, Taiwan and China. Although the genus is extremely common and certain of the taxa within the genus are among the most frequently encountered aquatic snails in the Orient, there is little useful biological information for adequately defining subgeneric categories. There has been great uncertainty in the interspecific systematics and species discrimination.

Over 30 species and subspecies of this genus have been described from Japan including the Ryukyu and Ogasawara (=Bonin) Islands. Kuroda (1963) lists most of these species in his catalog of the non-marine mollusks of Japan. The species definitely named or reported from the main Japanese Island of Honshu, the area from which most of the snails of this study came, are listed in Table 1. The plethora of names evidently resulted from describing local populations which are not only widespread in canals, rivers and lakes of Japan, but also exhibit great variability in shell size, shape, color patterns and sculpture.

Problems faced when working with this confusing and complex genus are many. Several of the major problems are: (1) any one "species" seems to grade into several others when adult shell features of numerous populations

TABLE 1. Species of *Semisulcospira* named or reported from the main island of Honshu, Japan*

Species group name	Original generic designation	Author and Publication
libertina	*Melania*	Gould, 1859, Proc. Bost. Soc. natur. Hist., 7: 42
*japonica***	*Melania*	Reeve, 1859, Conch. Icon., Monog. *Melania*, sp. 129, pl. 17, fig. 125
tenuisulcata	*Melania*	Dunker, 1860, Malakozool. Blätt., 6: 229
*rufescens***	*Melania*	Martens, 1860, Ibid., 7: 47
*ambidextra***	*Melania*	Martens, 1860, Ibid., 7: 46
*martensi***	*Melania*	Brot, 1862, Matér. Fam. Mélaniens, Cat. Syst. p 48
*reiniana***	*Melania*	Brot, 1876, Jahrb. Deut. Malakozool. Ges., 3: 277, pl. 8, fig. 4
niponica	*Melania*	Smith, 1876, Quart. J. Conch., 1: 124
libertina var. *decussata*	*Melania*	Martens, 1877, Sitz.-Ber. Ges. naturforsch. Freunde Berlin, 1877: 114
libertina var. *plicosa*	*Melania*	Martens, 1877, Ibid., 1877: 114
libertina var. *irrigua*	*Melania*	Martens, 1877, Ibid., 1877: 116
biwae	*Melania*	Kobelt, 1879, Senckenberg. Natur. Ges., 11: 132, pl. 19, fig. 9
niponica var. *decipiens*	*Melania*	Westerlund, 1883, Nachrichtsb. Deut. Malakozool. Ges., 15: 56
japonica var. *ornata*	*Melania*	Westerlund, 1883, Ibid., 15: 57
niponica var. *trachea*	*Melania*	Westerlund, 1883, Ibid., 15: 57
andersoni	*Melania*	Smith, 1886, J. Conchol., 5: 58
*mariesi***	*Melania*	Smith, 1886, Ibid., 5: 59
multigranosa	*Melania*	Boettger, 1886, Jahrb. Deut. Malakozool., 13: 7
yokohamensis	*Melania*	Hartman, 1897, Nautilus, 11: 41
reiniana var. *hidachiensis*	*Melania*	Pilsbry, 1902, Proc. Acad. natur. Sci. Phila., 54: 119, pl 9, fig. 2
libertina var. *latifusus*	*Melania*	Pilsbry, 1902, Ibid., 54: 120, pl. 9, fig. 8
libertina var. *gigas*	*Melania*	Pilsbry & Hirase, 1904, Nautilus, 18: 9
kawamurai	*Semisulcospira*	Kuroda, 1929, Venus, 1: 189, pl. 5, fig. 29-30
nakasekoae	*Semisulcospira*	Kuroda, 1929, Ibid., 1: 189, pl. 5, fig. 37-41
libertina nassaeformis	*Semisulcospira*	Kuroda & Kanamura, 1929, Ibid., 1: 188, pl. 5, figs. 25-26
decipiens reticulata	*Semisulcospira*	Kajiyama & Habe, 1961, Ibid., 21: 171, fig. 6
kurodai	*Semisulcospira*	Kajiyama & Habe, 1961, Ibid., 21: 173, figs. 1-3

* The list does not include nomen nuda or erroneous names. However, most of these nominal species are considered synonyms of *S. libertina* (Gould) (see Kuroda, 1963).

**Type locality, "Japan"; not definitely known from Honshu.

are studied; (2) Almost no morphological information has been added in recent years to that of the original papers, which described numerous species in few, brief paragraphs utilizing the "adult" shell alone, e.g., Gould (1859), Brot (1874), Kobelt (1879), Martens (1877), Westerlund (1883), etc. Morphological data on the soft anatomy of the genus are non-existent, except for the gross anatomy of *Semisulcospira libertina* (Itagaki, 1960), and Kajiyama & Habe's (1961) use of embryo shell characters in distinguishing *Semisulcospira* species. (3) There has been a recent trend to "lump" species, thus creating a list of synonyms without adequate justification or data. For example, Kuroda (1963) subordinated *Semisulcospira libertina* and 14 other named taxa to *S. bensoni* (Philippi, 1851). Most recently, Habe (1965) considered the genus *Semisulcospira* in Japan to be comprized of 4 species; *S. bensoni*, *S. niponica*, *S. kurodai* and *S. decipiens*. He reduced *S. multigranosa*, *S. nakasekoae* and *S. reticulata* to subspecies of *S. decipiens*.

Confusion will continue until an attempt is made to define basic genetic units within the genus. Characters must be brought forth which serve to objectively characterize taxa. Therefore, a program was initiated at the 406th Medical Laboratory to establish criteria for defining fundamental, biologically distinct groups within the genus. The basic approach was to locate topotype populations and study these using different methods and techniques. The populations sought and located were those of species which appeared quite distinctive in the original descriptions and figures. Data were correlated from cytological investigations (Burch & Davis, 1967; Burch, 1968) and studies on shell morphology, embryo shell characters and intra-brood pouch development patterns of the embryo shells.

The purpose of this paper is to present detailed descriptions of the 10 taxa of *Semisulcospira* from Honshu, Japan which appear to be very distinct. Two of the 10 taxa are new. The taxa were analyzed and compared using numerous characters previously unused or not used uniformly in collating the numerous named forms of *Semisulcospira* from Japan. The utility of different characters in defining taxa is discussed.

Of primary importance is the fact that topotype populations were used to establish expanded concepts of the species by studying hitherto unrecorded ranges of variability in characters. Within the framework of cytological findings it has been possible to untangle problems involving sibling species and polymorphic forms which had escaped the notice of early authors dealing with very limited segments of the type populations.

With an expanded central or core concept of the species based on topotype populations it is more easily possible to understand the wide range of variability exhibited by other populations. Populations may be relegated to a particular species group and trends in variability may be seen for a number of characters and how these fit into an overall unified species concept.

II. MATERIALS AND METHODS

Living specimens were collected in large numbers, generally 300 to 1000 of each shell type from each population studied, in order to establish limits of variability within populations. In a few cases, a given population yielded few individuals, 3 to 20 of a desired shell type. Specimens were split into 3 groups when large numbers were available; each group had the same composition of size range of individuals. One group was placed in 70% alcohol, another was placed in culture in the laboratory, and the 3rd was boiled in commercial Clorox (5.25% sodium hypochlorite). Living adults maintained in the laboratory were used for the cytological studies mentioned previously.

Fully adult animals were desired to

obtain adequate estimates of young in the pallial brood pouch as well as adult shell characteristics and upper limits of shell size. Abbott (1952) used the largest 10% of each population in his study of *Thiara granifera*, because there was no distinctive way of telling when a snail had reached full limits of growth. He stated, "Since growth is continuous throughout the life of the individual, two factors will delimit the length of the shell - genetic and environmental." I also chose the largest 10% of the populations to characterize each taxon in terms of adult characters and to obtain embryonic shells. Measurements and observations involving adult snails were made using a series of snails selected at random from the largest 10%. The selection of an arbitrary cut-off point of 10% will be discussed later.

In almost every population a number of apical whorls were eroded from the adults. Therefore, measurements of length of shell are limited in application. Size is best discussed in terms of length of body whorl and shell width. I used the length of the body whorl to determine which specimens were in the largest 10% of the population.

All shells, adult or embryos, were treated with Clorox when only the shell characters were to be studied (a valuable method of Walter, 1962). Since adult shells were frequently covered with algae or caked with hard, black deposits, such treatment aided in removal of the periostracum with adhering artificial deposits, and all animal remains. This treatment left a perfectly clean shell where all details of sculpture, shell banding and true color could be clearly seen.

Lengths and widths of shells were recorded along with spire increment angles, numbers of whorls, ribs and basal cords, lengths and widths of apertures, lengths of body whorls, presence or absence of nodes on the ribs and shell color patterns. Methods of measuring several of these characters are presented below.

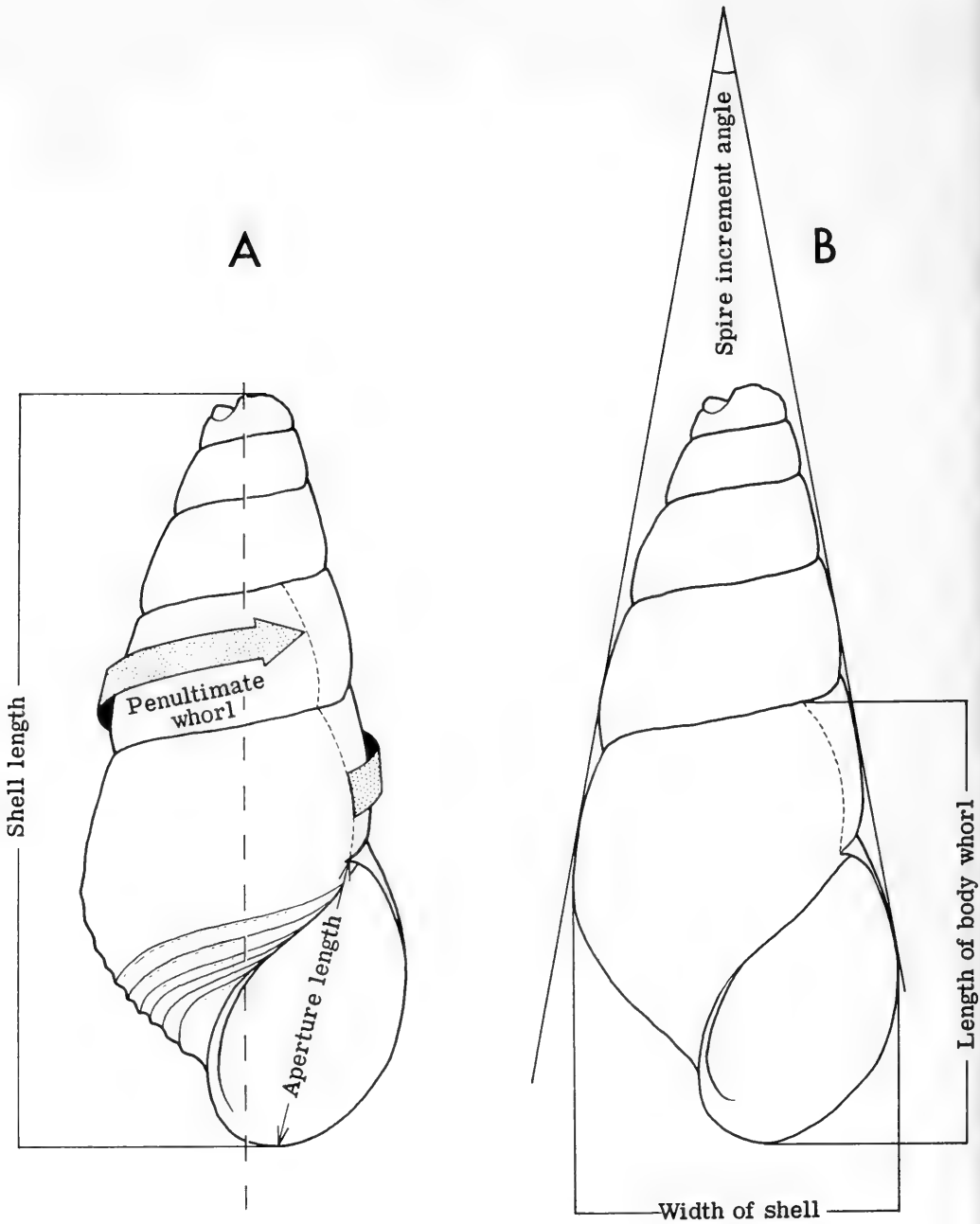
Adults

Shell measurements. A compass (divider) was used in conjunction with a ruler marked off in mm. Measurements were accurate to the nearest whole mm and were rounded off to the nearest 0.5 mm. Shell features measured are illustrated in Text Fig. 1. The length of aperture was measured at its greatest length, and was not measured parallel to the shell axis. The length is the hypotenuse of a triangle which has its base perpendicular to the shell axis (Text Fig. 1A).

Spire increment angle. Throughout the paper, the term spire increment angle is shortened to spire angle. As shown in Text Fig. 1B, straight lines touching the body whorl, penultimate whorl, and those other adapical whorls which meet the lines, were extended to a juncture and the angle resulting from the 2 intersecting lines was measured. Generally the apical whorls do not touch the lines, thereby indicating an incremental change in angle as the specimens grow older. Measurements were accurate to the nearest full degree.

Ribs. Unless specified, ribs were counted on the penultimate whorl, as were the nodes and cords which sometimes crossed the ribs. Often the body whorls were devoid of sculpture, the sculpture was very weak, or the ribs were imperfectly formed near the outer lip.

Basal cords. As shown in Text Fig. 1A, basal cords are those arising on the body whorl at the adapical tip of the aperture down to the shell base. Cords spiraling anteriorly from the penultimate whorl at the suture and passing just past the adapical tip of the aperture may be pronounced, but they were considered to belong to the cords counted on the penultimate whorl. Basal cords were counted at magnifications of 6× and 16×.



TEXT FIG. 1. Diagrams illustrating methods of making adult shell measurements of Japanese *Semisulcospira* species.

Embryos

The study of embryonic shells involved establishing the number of young per pallial brood pouch, i.e., young per female, and finding the percentage of young at each whorl stage. In addition, shell lengths and widths, as well as lengths of body whorls were measured, and the points on the apical whorls where ribs first started, as well as the number of ribs per first ribbed volution, were recorded. Also, features of shell sculpture and the color patterns of the shells were analyzed.

To obtain the young, specimens were chosen at random from the largest 10% of the populations which had been preserved in alcohol. The individual adult shells were cracked off and the pallial brood pouches were dissected out entire, care being taken not to lose any of the embryos. The individual brood chambers were placed in separate vials filled with Clorox. This resulted in the oxidation of all tissues and eggs, leaving only the shells from the earliest to the later stages. The cleaned embryonic shells were washed in alcohol and stored in clear water (slightly basic) until they were studied.

Embryos were studied under the dissecting microscope at magnifications of 16× and 40×. Measurements were made with a standard ocular micrometer and were accurate to 0.024 mm.

Whorls were counted, as discussed by Davis (1967a), to the nearest 0.5 whorl. Shells appearing to be an intermediate +.25 or +.75 whorls were not used for measurements. Embryos were not studied or measured from just 1 female of a taxon, but from as many as possible.

III. HABITATS AND FAUNAL ASSOCIATIONS

Collection sites are shown in Text Figs. 2-4. I attempted to obtain as many topotypes as possible from the Lake Biwa region because this area is noted for its high endemicity and because several species of *Semisulcospira* described from this area appeared to be quite distinct.

Station 1 (Text Fig. 2). Topotypes of *Semisulcospira niponica.*

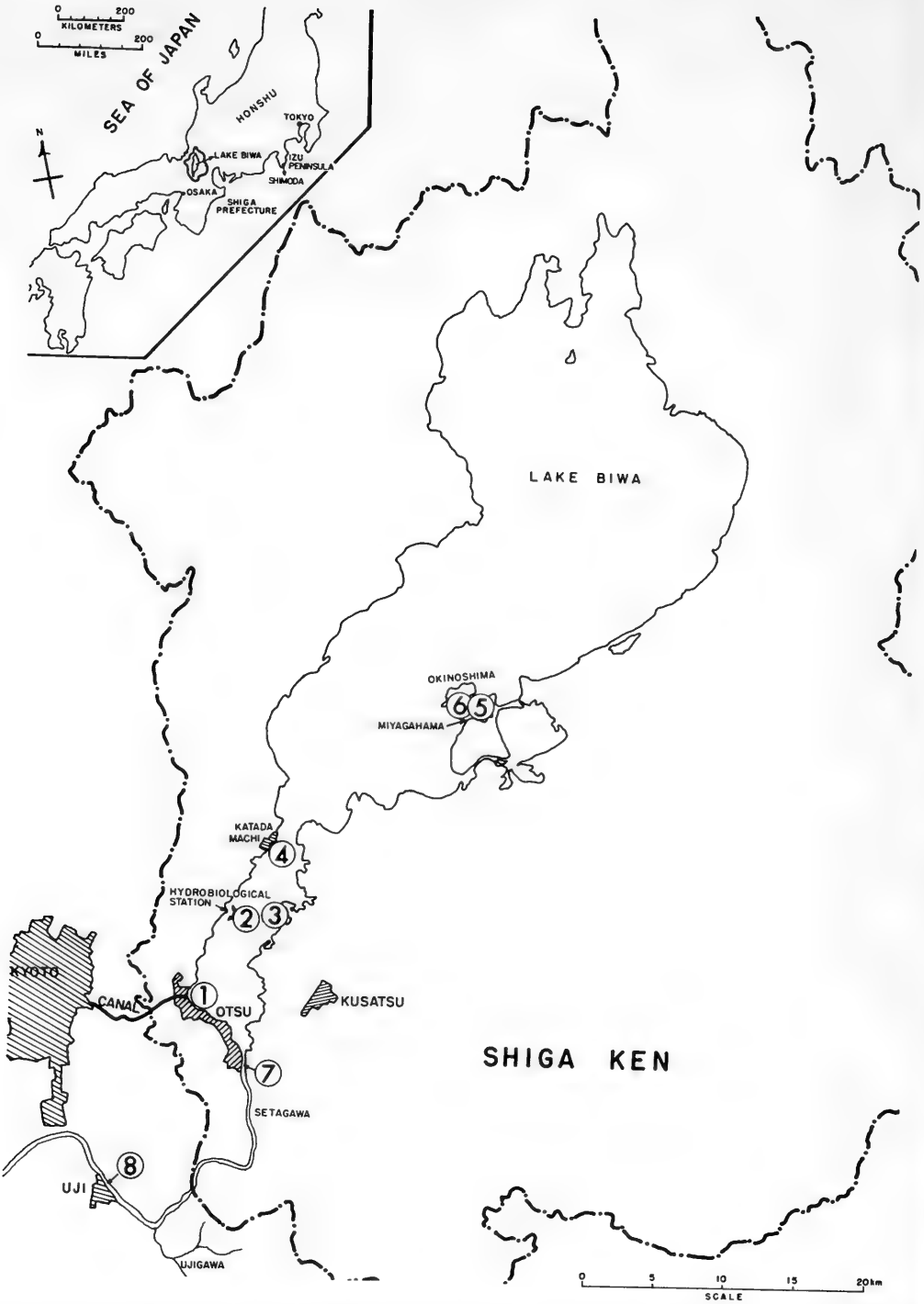
Shiga Prefecture, Otsu City, 24 July 1965. At this locality a spit of land projects into Lake Biwa. On one side of the spit is the canal leading from the lake to Kyoto; on the other side a waterway, blocked at intervals by a series of locks, passes into the city. Snails were collected from the stone support of a bridge crossing this waterway where it leads from the lake. Snails were numerous in the shallows to a depth of 0.6 meter on rocks as well as the bridge supports. Mollusks collected were: *Semisulcospira niponica* and *Sinotaia histrica.*

Station 2 (Text Fig. 2).

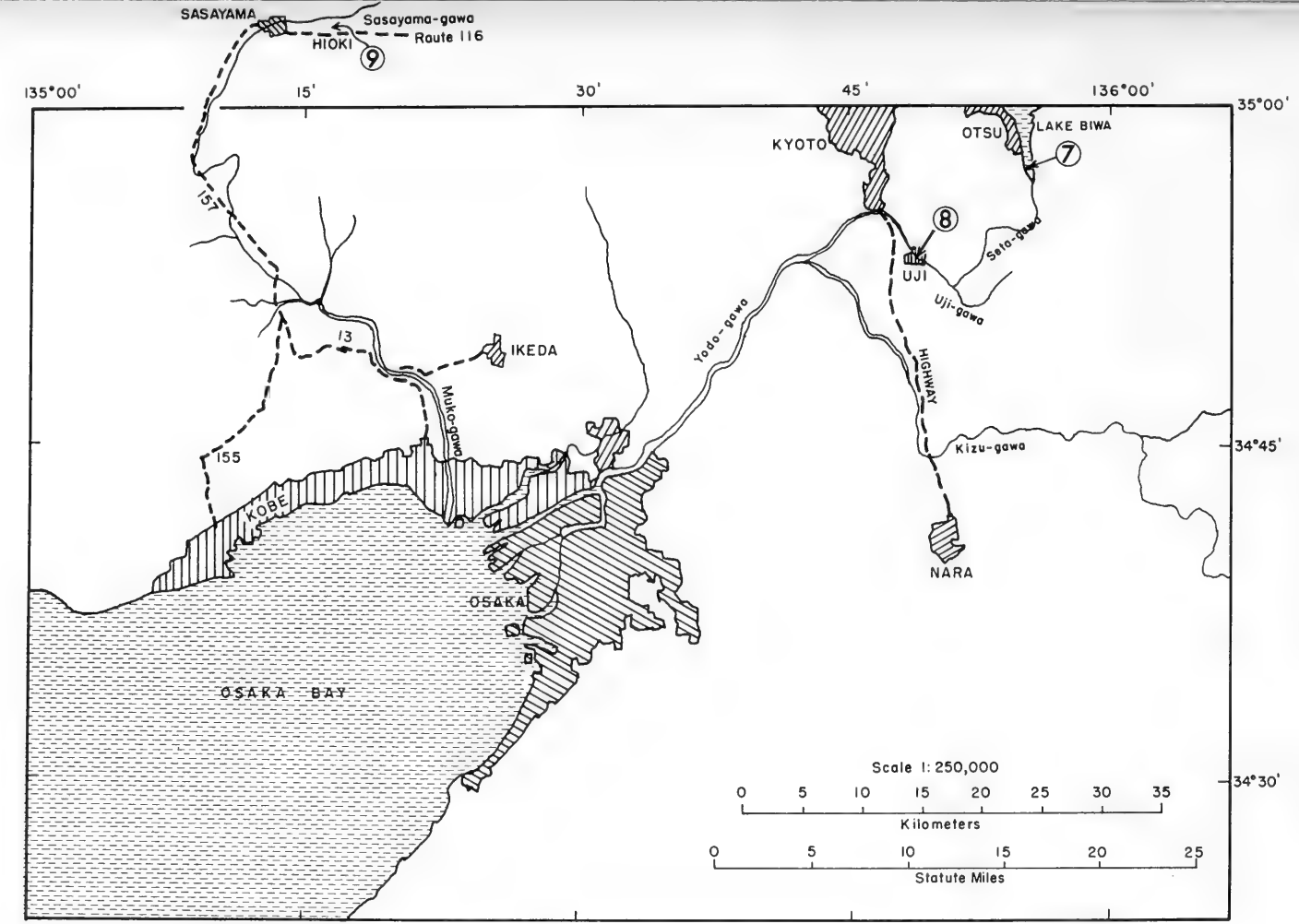
Shiga Prefecture, Otsu City, from a stretch of Lake Biwa ¼ mile off shore (from Shimo-sakamoto to Shin-karasaki sections of Otsu), 23 July 1965 and 8 November 1965. The following mollusks were dredged from a depth of about 4 meters where the bottom was claymud with no vegetation: *Semisulcospira reticulata* (dominant), *Heterogen longispira*, *Unio biwae*, *Lanceolaria oxyrhyncha*, *Anodonta calipygos*, *Inversidens brandti*, and *Corbicula sandai.*

Station 3 (Text Fig. 2). Types of *Semisulcospira habei yamaguchi;* Topotypes of *S. decipiens.*

Shiga Prefecture, north of Shina-naka harbor off Kusatsu City, 23 July 1965 and 8 November 1965. The shelly-mud bottom was dredged at a depth of 3 meters. Vegetation was sparse. Mollusks collected were: *Semisulcospira decipiens* (dominant), *S. habei yamaguchi*, new subspecies (sparse), *S. multigranosa* (smooth form), *Corbicula sandai* (dominant), *Unio biwae* (dominant),



TEXT FIG. 2. Map of Shiga Prefecture and surrounding area showing collecting stations in and near Lake Biwa. The heavy broken line shows the limits of the prefecture. The location of this prefecture on Honshu I. is shown in the inset.



TEXT FIG. 3. Map of the area southwest of Lake Biwa showing collecting stations 7, 8 and 9.

Inversidens reiniana, *I. brandti*, *Anodonta calipygos*, and *Lanceolaria oxyryncha*.

Station 4 (Text Fig. 2).

Shiga Prefecture, Katada Town, fish pier and beach, 24 July 1965 and 10 November 1965. Shells were taken from large shell mounds at the beach area of the town. Sub-fossils of *Semisulcospira reticulata* were obtained along with shells of 9 other species on 23 July 1965. On 8 November 1965, living *S. reticulata* were taken from the bottoms of fishing boats where they were cast aside. These most probably came from several widespread areas around Katada and were dredged up by fishermen interested in obtaining *Heterogen longispira*, *Corbicula sandai*, or *Hyriopsis schlegeli*. The last species is used in the pearl industry.

Station 5 (Text Fig. 2). Topotypes of *Semisulcospira reticulata*.

Shiga Prefecture, Lake Biwa, off Okino-Shima, between the island and Miyagahama on the mainland, 10 November 1965. Typical *Semisulcospira reticulata* were collected from a hole 6-7 meters deep. The bottom was devoid of vegetation.

Station 6 (Text Fig. 2). Topotypes of *Semisulcospira multigranosa*.

This locality is the same area as Station 5, but the depth was about 4 meters and the lake bottom was covered by dense vegetation. The vegetation faded out only in the shallows at the shore of the mainland where the bottom was sandy. Mollusks dredged up were: *S. multigranosa* (ribbed and smooth), *S. habei yamaguchi*, *S. niponica* and *Heterogen longispira*.

Station 7 (Text Figs. 2 and 3).

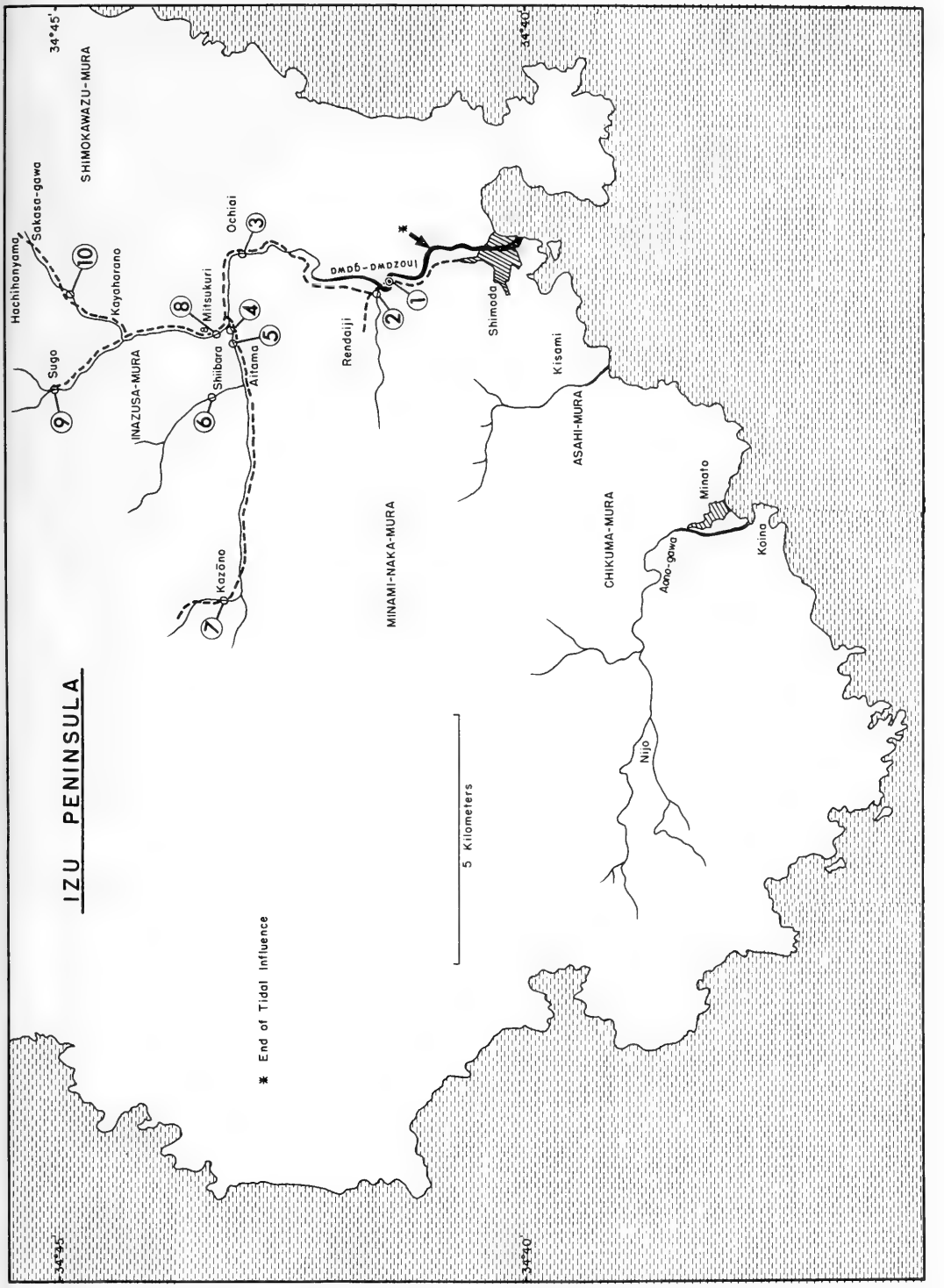
Shiga Prefecture, mouth of Seta River, 9 November 1965. Four specimens were found in about 30 cm of water at the banks of an island in the mouth of the river. Three of these were *Semisulcospira habei yamaguchi*, the 4th was *Radix onychia*.

Station 8 (Text Figs. 2 and 3). Types of *Semisulcospira habei;* Topotypes of *S. nakasekoae*.

Kyoto administrative district, Uji City, Uji River, 24 July 1965 and 9 November 1965. Snails were collected from both sides of the river opposite the railway station of Uji and from the banks and on stones in the rapids at the eastern edge of Togashima, an island in the middle of the river. Topotypes of *Semisulcospira nakasekoae* were collected from the rocks in the rapids and from rocks in the shallow quiet water of the western bank of the river. *S. habei*, new species, was collected from rocks and the embankment of the eastern shore of the island. A few specimens of *S. reiniana* came from the island area, but most were obtained on the rocks and the walls containing the east bank of the river. The Uji River at this locality was subject to marked changes of water level with corresponding changes in current. Following heavy rains, the river rapidly becomes a raging torrent.

Station 9 (Text Fig. 3). Topotypes of *Semisulcospira kurodai*.

Hyogo Prefecture, Jyoto-cho, 6 August 1965 and 16 January 1966. *Semisulcospira kurodai* was collected from a small drainage ditch behind the home of Dr. Tadashige Habe, co-author of the species. The ditch was about 100 yards from the main road running from the town of Sesayama through the area called Hioki. The ditch was close to the tracks of the Japanese National Railway running to Sesayama and Sesayama-guchi. Sympatric with *S. kurodai* at this locality is *S. libertina*. These 2 species were found burrowing in the silt, covered by shallow, gently flowing water. *S. kurodai* was also found in the ditch alongside the highway which runs through the town. Passing over the tracks, away from the highway and towards the Sesayama River, is another drainage



TEXT FIG. 4. Map of the tip of the Izu Peninsula, Shizuoka Prefecture, showing the presumed type locality of *Semisulcospira libertina* at Shimoda.

ditch parallel to the above. This ditch contained many *S. libertina* and only a few (3 in over 100 snails collected) *S. kurodai*. The same 2 species were collected in the shallows of the Sesayama River, in quiet water on stones and in thin pockets of silt.

Station 10 (insert, Text Fig. 2; Text Fig. 4). Topotypes of *Semisulcospira libertina*.

Shizuoka Prefecture, Shimoda Town, Inozawa Section, Inozawa River, 12 August 1965. Gould (1859) did not designate a type locality when he described *Semisulcospira libertina* as occurring both at Shimoda on the main island of Honshu, and Amami-oshima (Ousima), an island of the Ryukyu chain. Yen (1944) published a picture of a specimen from a lot of 3 specimens marked Type C, No. 2120 from Amami-oshima. He stated that another lot of 2 specimens was from Shimoda. Johnson (1964) chose as lectotype the specimen figured by Yen from Lot 2120, but stated that 2 specimens from Shimoda were paratypes (Lot 42-1, Redpath Museum ex Smithsonian Institution).

I located the population at Shimoda which is the one most likely sampled by Stimpson who collected the type series of *Semisulcospira libertina*. The Inozawa River empties into the harbor of the small fishing village of Shimoda on the Pacific Ocean. The village, habor and river are in a deep valley, hemmed in on either side by mountains. In quest of freshwater snails from Shimoda, one must go up the river to a point beyond the tidal influence. A short distance above this point I found not only specimens of *S. libertina*, but a very large population of this species. The population was under and about an old bridge called Hongo-bashi (Site 1, Text Fig. 4). The road crossing this bridge is the main road inland from Shimoda and it parallels the river.

Although I had material collected from Amami-oshima, I was not able to identify any one population from this large island as representing the type population.

Mollusks collected with *Semisulcospira libertina* at Shimoda were: *Clithon retropictus* (dominant) and *Physa* sp. (few). *S. libertina* was found scattered throughout the drainage system of the Inozawa River. Stations from which the species was collected are shown in Text Fig. 4.

Station 11. Topotypes of *Semisulcospira libertina*.

Ryukyu Island chain, Amami-oshima, Nase City, Yamato village and Koshuko village, 29 October 1965. Snails were collected from rice paddies and ditches in the villages of Nase City by members of the Department of Entomology, 406th Medical Laboratory, as follows: *Semisulcospira libertina*, *Thiara scabra*, *Radix japonica* and *Melanoides tuberculatus*.

IV. DESCRIPTION AND ANALYSIS OF SPECIES

In this section, 10 distinct taxa are described using characters which are, for the most part, listed in Table 41 (p 269). Each taxon is treated as a unit complete with descriptions and tables. Data from the tables are used in later sections dealing with (1) comparisons of taxa in terms of growth patterns of embryos (p 262 and Text Figs. 5 to 10); (2) comparisons of taxa using traits considered to be of primary, secondary or tertiary importance (p 268 and Text Figs. 5 to 19); (3) a key to the species using the afore mentioned characters (p 270).

At the end of each species description the taxon is contrasted with those species with which it is most likely to be confused. Each taxon belongs to one of 2 species groups as discussed on pages 272 and 279. Species recognition is aided by the key (p 270), the discussion in the section on the utility of characters (p 272) and the use of Text Figs. 11 to 19.

Semisulcospira libertina
(Gould, 1859)

Shimoda Station (No. 10) - Gould's type locality (in part).

Adults (Pl. 1, Figs. 1-3)

An analysis of variation in fundamental shell features is given in Table 2. The sides of the whorls are flat to slightly convex. The most anterior 3 or 4 basal cords are the most prominent of the spiral cords on the body whorl. The middle part of each whorl is usually smooth and a complete complement of 8-11 cords (or grooves) is distinct on the penultimate whorl in about 10% of the shells. One or 2 spiral grooves are comparatively prominent just below the suture of each whorl. Ribs are present in only 3% of the population. When present, there are 12-18 ribs per penultimate whorl, usually in the form of low swellings (Pl. 1, Figs. 2, 3). In only 0.5% of the population are cords and ribs prominent enough to create a nodulate appearance on the ribs (Pl 1, Figs. 2, 3).

As shown in Table 3, none of the shells had a solid dark color. The uniformly colored shells varied from light yellow (very common) to light brown (extremely rare). The body whorl often appeared light yellow while the adapical whorls were darker yellowish-brown. In the banded shells (39.5%), 3 types of pattern were found (Table 3): 1 band, which occurs either between the suture and the adapical tip of the aperture or starts at the mid-parietal wall and spirals anteriorly; 2 bands (Pl. 1, Fig. 1); 3 bands (Pl. 1, Fig. 2). The 3 bands shown in Pl. 1, Fig. 2 are the basal, mid-whorl and subsutural bands; they are purple brown.

Adult shells (inevitably eroded) usually had 4 whorls, a few had 5. The average length of the body whorl was 19.2 mm. Where the body whorl varied from 18.7 to 19.7 mm, the average aperture length and width were 12.8 and 7.3 mm, respectively, with a length/width ratio of 1.75.

Embryos (Pl. 8, Figs. 1-5)

A statistical analysis of numbers of young per pallial brood pouch along with the percentages of young at each stage is presented in Table 5; included are statistics on shell measurements. Complete statistics of embryo shell measurements are given in Appendix 1. The young shells do not have nodes or ribs. The embryos are small (Table 38, p 260) and elongate (Table 40, p 266). Most have 1 or 2 spiral cords (Table 4; Pl. 8, Figs. 2, 3). The presence of cords increases with increased whorl stages as seen in Table 4. The adapical cord passes between the suture and the adapical tip of the aperture. The abapical cord is pronounced only on the body whorl, if present at all. The outer lip is distinctly notched at the termination of the cords. They are generally brown, but many vary from straw-yellow to brown.

Microsculpture on the fragile shells is pronounced at magnifications of 16 and 40X. The pattern is cancellate owing to micro-threads crossing growth lines. That part of the shell bordering the columella is often purplish, and in a few cases, a purple band is formed.

Whorls are quite convex with sutures correspondingly impressed. The tip of the apex is depressed below the succeeding ¼ whorl. See Appendix 2 for measurements of the apical whorl.

Amami-oshima Station (No. 11) - Gould's type locality (in part).

Adults (Pl. 1, Fig. 4)

Fundamental shell features of this population are compared with those of the Shimoda population in Table 2. The most anterior 3 or 4 basal cords of the spiral cords on the body whorl are the most prominent. There are 11 ± 1 distinct spiral cords on the penultimate whorl. In 20% of the shells the cords are separated by 8 to 10 distinct spiral grooves. Shells are uniformly straw-

PLATE 1. Adult shells of 2 species of *Semisulcospira*. The measurement line is in mm.

FIGS. 1–3. *Semisulcospira libertina* (Gould) from Shimoda. Note faint ribs on the specimen in Fig. 1.

FIG. 4. *Semisulcospira libertina* (Gould) from Amami-oshima. The spiral cords are more pronounced than in specimens of the same species from Shimoda.

FIGS. 5, 6. *Semisulcospira reiniana* (Brot).

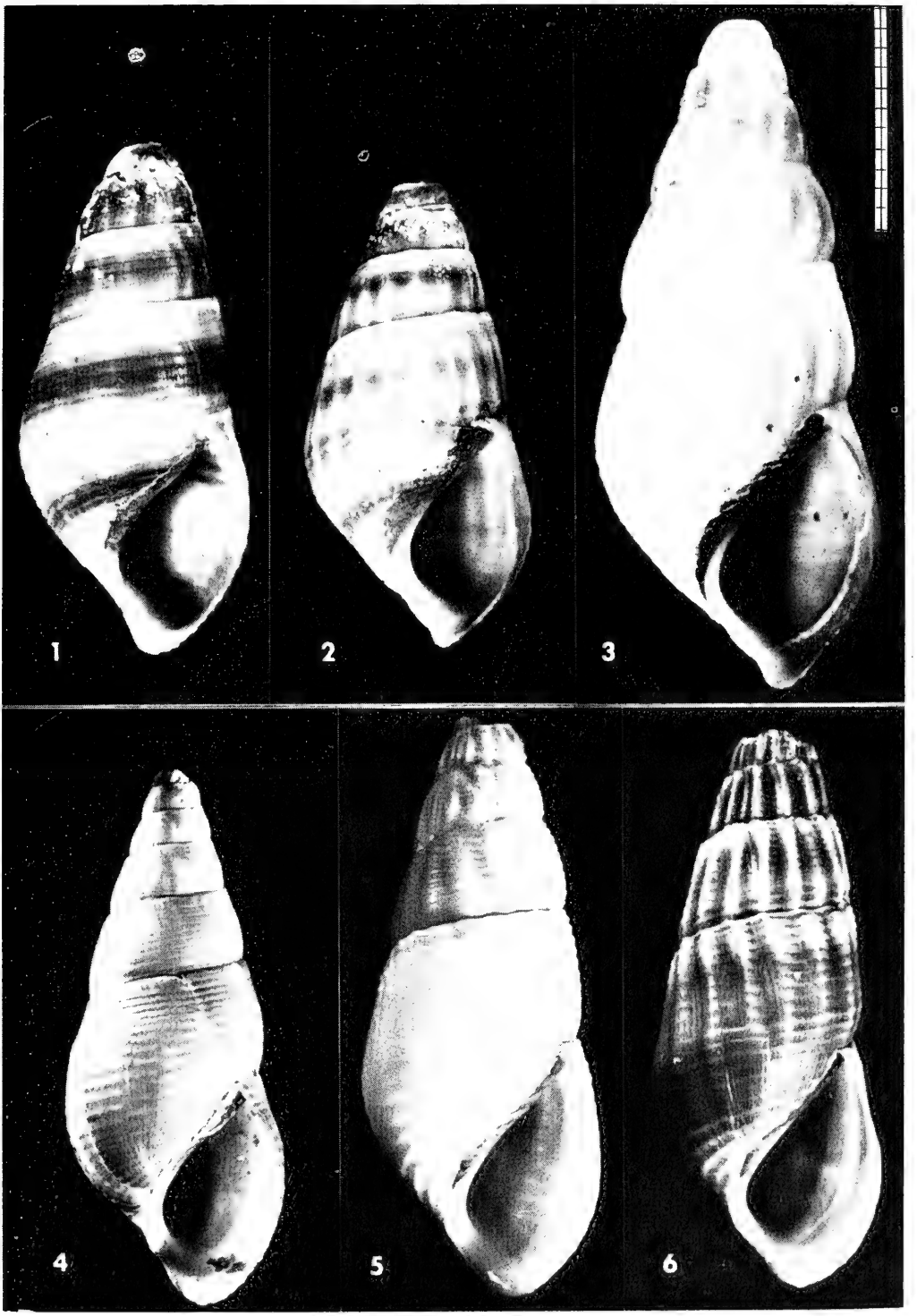

1
2
3
4
5
6

TABLE 2. Adult shells; a statistical analysis of shell features of *Semisulcospira libertina*

Locality	Number specimens examined	Statistic	Feature measured or counted					
			Spire angle	Ribs	Basal cords	Body whorl length (mm)	Shell length (mm)	Shell width (mm)
Shimoda	25	$\bar{X}$	22.2°	*	9.2	19.2	28.6	13.0
		S	3.1		1.6	1.7	--	1.3
		Se	0.63		0.31	0.33	--	0.26
Amami-oshima	25	$\bar{X}$	22.6	0	9.5	16.3	26.0	11.0
		S	2.3	—	1.3	0.9	--	0.7
		Se	0.46	--	0.03	0.19	--	0.15

* see text

$\bar{X}$ = Mean
S = Standard deviation
Se = Standard error of the mean

TABLE 3. Color patterns of adult *Semisulcospira libertina*

Locality	Number specimens examined	Color pattern (% of shells)				
		Uniform yellow	Uniform purple	1 band	2 bands	3 bands
Shimoda	200	60.5	0	9.0	15.5	15.0
Amami-oshima	50	100	0	0	0	0

TABLE 4. The number and frequency of occurrence of cords on embryos of *Semisulcospira libertina* at each whorl stage

Locality	Whorl stage	No. embryos examined	% of shells		
			0 cords	1 cord	2 cords
Shimoda	2.0	1271	24.3	47.0	28.7
	2.5	629	10.2	29.1	60.7
	3.0	27	3.7	25.9	70.4
Amami-oshima	2.0	441	27.9	72.1	0.0
	2.5	101	8.9	91.1	0.0
	3.0*	--	--	--	--

* insufficient embryos for study

yellow (Table 3); no banded shells were found.

Adult shells (eroded) had 5 ± 1 whorls. The average length of the body whorl was 16.3 mm. Where the body whorl varied from 18.7 to 19.7 mm, the average length and width of their apertures was 11.1 and 6.4 mm, respectively, with a length/width ratio of 1.73.

Embryos (Pl. 8, Figs. 6-10)

A statistical analysis of the numbers of young per pallial brood pouch, along with the percentages of young at each whorl stage, is presented in Table 5, including statistics on shell measurements. Apical whorl measurements are given in Appendix 2. Complete statistics of embryo shell measurements are given in Appendix 1.

Embryonic shells are similar to those described from females at Shimoda, with the exception of details concerning cords, apical whorl measurements and microsculpture. None of the shells had 2 cords (Table 4). The single cord becomes more evident on shells of 2½ to 3 whorls. The apical whorl has an average diameter of 0.47 mm, which is significantly smaller than that of the Shimoda snails (P = 0.01). The tip of the apex is either emergent or suppressed below the following ¼ whorl. The microsculpture is limited to growth lines (at magnification of 16 and 40X). The distinct spiral threads described for the embryos from the Shimoda population are absent.

Comparison of species

Semisulcospira libertina may be confused with *S. reiniana*. The populations of the former which have nodulate ribs (see p 270) appear very much like adult *S. reiniana*. The taxa differ in that the former has a chromosome number of n=18 while the latter has n=20. The embryos of the former are very significantly smaller than are those of the latter (Table 38). It is evident in Text Fig. 5 that the taxa differ in the slope of the curve for shell length per whorl. Embryos of *S. libertina* are smooth or with nodes (p 274), while those of *S. reiniana* have pronounced ribs. Further differences are evident in Text Figs. 7 and 9 involving growth characteristics of the embryos.

Semisulcospira reiniana (Brot, 1876)

Station 8

Adults (Pl. 1, Figs. 5 & 6)

Statistics on fundamental shell features are given in Table 6. The most anterior 3 or 4 basal cords of the spiral cords on the body whorl are especially pronounced. Ribs vary in prominence from low to highly folded pleats; they are nodulate where they are crossed by spiral cords. The suture is scalloped where the whorl curves around the rib of the adjacent adapical whorl. The ribs tend to fade out on the body whorl near mid-whorl (indicating full adult status); they are not prominent for about ½ whorl back from the aperture.

There are 11.0 ± 1.66 (mean and standard deviation) spiral cords on the penultimate whorl. In 8% of the population raised cords are not as pronounced as inter-cord grooves, of which there are 8-11 on the penultimate whorl.

As seen in Table 7, there are 5 color patterns. Generally, when the shell is uniform blue or blue-purple, 2 bands, the apical one the wider, can be seen on the inside of the outer lip. When the external shell has 1 band, it is generally found at mid-whorl. When 2 purple bands are seen they are separated by a narrow yellow belt above mid-whorl. In the case of 3 bands, in addition to the subsutural band, the wide basal band (of the 2-banded condition) is divided by a yellow belt.

Following the shell coil back from the aperture one observes that the bands do not become evident for ⅕ to ⅓ of a volution. The yellow of the lip edge protrudes between the 2 fading bands as a V-shaped wedge or squarish notch. Spiral cords stand out white against the

TABLE 5. Embryo shells; a statistical analysis of numbers per female and shell features of *Semisulcospira libertina*

Locality	Number females examined	Number young per female	Feature studied	Mean measurements (mm) of embryos of different whorl counts				
				<2.0	2.0	2.5	3.0	3.5
Shimoda	11	$\bar{X}$, 351.6	Shell length		0.95	1.16	1.35	--
		S, 241.7	Shell width		0.74	0.84	0.93	--
		Se, 72.9	Body whorl L.		0.78	0.90	0.99	--
			Ratio L/W		1.28	1.38	1.45	
		Range 168-979	% total embryos	47.6	35.6	16.1	0.7	
Amami-oshima	11	$\bar{X}$, 229.4	Shell length		0.92	1.18	1.39	--
		S, 84.4	Shell width		0.74	0.84	0.99	--
		Se, 25.5	Body whorl L.		0.78	0.96	1.08	--
			Ratio L/W		1.24	1.40	1.40	
		Range 137-384	% total embryos	76.6	18.3	4.9	0.2	

$\bar{X}$ = mean
S = standard deviation
Se = standard error of the mean
L = length
W = width

TABLE 6. Adult shells, a statistical analysis of shell features of *Semisulcospira reiniana*

No. specimens examined	Statistic	Feature measured or counted					
		Spire angle	Ribs	Basal cords	Body whorl length (mm)	Shell length (mm)	Shell width (mm)
25	$\bar{X}$	20.6°	16.0	8.3	17.9	26.1	11.1
	S	3.36	1.63	1.23	1.43	--	0.74
	Se	0.67	0.33	0.25	0.29	--	0.15

$\bar{X}$ = mean
S = standard deviation
Se = standard error of the mean

TABLE 7. Color patterns of adult *Semisulcospira reiniana*

Number specimens examined	Uniform blue or purple-blue	Uniform yellow	1 band	2 bands	3 bands
110	21.8%	26.4%	1.8%	36.4%	13.6%

TABLE 8. Embryo shells; a statistical analysis of numbers per female and shell features of *Semisulcospira reiniana*

Number females examined	Number young per female	Feature studied	Mean measurements (mm) of embryos of different whorl counts				
			< 2.0	2.0	2.5	3.0	3.5
10	$\bar{X}$, 138.0	Shell length		0.93	1.28	1.64	1.89
	S, 53.0	Shell width		0.78	0.97	1.19	1.29
	Se, 16.8	Body whorl L.		0.79	1.03	1.21	1.37
		Ratio L/W		1.19	1.32	1.38	1.47
	Range 74–225	% total embryos	[<2.5 (57.5)]		23.9	14.3	4.3

$\bar{X}$ = mean
S = standard deviation
Se = standard error of the mean
L = length
W = width

TABLE 9. Color types of *Semisulcospira reiniana* embryos from 10 females

Embryos from each female		% of females	Color of embryos	% of embryos
All embryos 1 color		30	yellow	33
			brown	67
Embryos with 2 color types	embryos predominantly yellow	70	yellow	68
			brown	32
	embryos predominantly brown		yellow	29
			brown	71

dark bands or uniformly dark shells. This gives the ribs a white-nodulate appearance.

Shells were predominantly 4 whorled (eroded) although a few had 3 or 5 whorls. The average shell length and width was 26.1 and 11.1 mm, respectively. The body whorl averaged 17.9 mm in length. Where the length of body whorl varied from 17.4 to 18.4 mm, the average aperture length and width were 11.7 and 6.3 mm respectively, with a length/width ratio of 1.85.

Embryos (Pl. 8, Figs. 11-15)

Statistics on numbers of young per pallial brood pouch along with the percentage of young at each whorl stage are presented in Table 8; included are statistics on shell measurements. Data of use for performing analyses of variance of shell measurements are given in Appendix 1. Measurements of the apical whorl are given in Appendix 2.

Shells reach 3.5 whorls in the brood pouch, are predominantly ribbed and have a pronounced cord at mid-whorl. They are medium sized (Table 38, p 260) and elongate (Table 40, p 266). Ribs (Appendix 2) first appear at 2¼ to 2½ whorls. In 2% or less, ribs appear as early as 1¾ whorls or as late as 3¼ whorls. Early ribs have a diameter of 0.12 - 0.17 mm; they are more pronounced at mid-whorl where they are thicker and jut out more. Ribs at mid-whorl generally have pronounced nodes. Twenty per cent of the shells of 3 or more whorls lacked ribs.

On dark shells, the cords stand out as a darker purple-brown. On lighter shells the cords are slightly darker than the background color. The frequency of occurrence of cords on embryos of different whorl stages is given in Table 10. Two spiral cords are more in evidence in shells of 2½ whorls than in those of later whorl stages. The first indication of ribs is generally seen as nodes on the adapical cord. This adapical cord at the whorl shoulder disappears at 3 to 3½ whorls. The cord at mid-whorl on the body whorl may be nodulate owing to the termination of ribs on this cord. In a few cases (in shells of 3.0 - 3.5 whorls) nodes on the ribs, 0.16 to 0.17 mm above the cord at mid-whorl, are connected by a cord.

Two classes of shell color were evident; (1) brown to dark reddish-brown, (2) light straw yellow to light yellow-brown. Banding patterns on the embryos were not found. In the light shells, the apical 2 whorls were generally yellowish-brown with later whorls much lighter. In light colored shells a purple patch twisted along the columella at the shell base. As shown in Table 9, all young from each female in 30% of the females were of a uniform color (brown or yellow) while young from each female in 70% of the females were mixed in color. When the embryos were mixed in color, the ratio was about 3 to 1, with one or the other color predominant. Where young from a female were uniform in color, 2/3 of the cases had dark embryos. Shells are fragile and the tip of the apex is emergent or suppressed.

Comparison of species.

See the section under *Semisulcospira libertina*.

Semisulcospira kurodai Kajiyama & Habe, 1961

Station 9, Topotypes

Adults (Pl. 2, Figs. 1-3)

Statistics on basic shell features are given in Table 11. The most anterior 2 to 4 basal cords of the spiral cords on the body whorl are generally the most pronounced. The shells are fragile and smooth with ribs occurring on only the 1 or 2 most apical whorls of the adult. When erosion of the spire of the adult shell is minimal, ribs are seen (Pl. 2, Figs. 1, 2) and these average 15 (standard deviation of 1.75 and standard error of the mean of 0.49) on the whorl where they begin to fade out. On this whorl the ribs measure 0.39 -

TABLE 10. The number and frequency of occurrence of cords on embryos of *Semisulcospira reiniana*

Whorl stage	No. embryos examined	% of shells		
		0 cords	1 cord	2 cords
2.5	25	8	48	44
3.0	25	4	76	20
3.5	25	0	80	20

TABLE 11. Adult shells; a statistical analysis of features of *Semisulcospira kurodai*

No. specimens examined	Statistic	Feature measured or counted					
		Spire angle	Ribs	Basal cords	Body whorl length	Shell length (mm)	Shell width (mm)
25	$\bar{X}$	16.6	see text	5.1	15.2	25.6	9.1
	S	3.08		1.02	0.89	--	0.60
	Se	0.62		0.20	0.18	--	0.12

$\bar{X}$ = mean
S = standard deviation
Se = standard error of the mean

TABLE 12. Color patterns of adult *Semisulcospira kurodai*

Number specimens examined	Uniform white or pale yellow	Uniform dark color	1 band*	2 bands**	3 bands***
73	15%	0	17.8%	15.1%	52.1%

* of the 17.8%, 15.0% was type 2 banding, 2.8% was type 5 (see text).
** of the 15.1%, 12.3% was type 3 banding, 2.8% was type 4 (see text).
*** of the 52.1%, 49.3% was type 1 banding, 2.8% was type 6 (see text).

0.43 mm in diameter; the whorl has an average mid-whorl diameter of 5.0 mm (with a standard deviation of 0.65 and a standard error of the mean of 0.18). The ribs rise rather abruptly from the shell surface.

Shells of this species are remarkably smooth abapical to the region of ribs. In about 56% of the population, 1 to 3 faint cords were observed just abapical to the suture (at magnifications of 5-6 ×).

As seen in Table 12, 85% of the shells were banded. The basic shell color varied from white to pale yellow. The bands varied from brown to purple-brown. Six banding patterns were encountered. (1) 3 bands (basal, mid-whorl, subsutural) as shown in Pl. 2, Fig. 1. The subsutural band was usually a very thin brown one. Often the bands at mid-whorl and base were weak and interrupted. (2) An indication of a spiral streak of brown was observed here or there on the shell at mid-whorl, subsutural area, or base. (3) Subsutural and basal bands only were found. (4) Only the subsutural and mid-whorl bands were found. (5) A distinct band at the suture spiraled anteriorly onto the mid-body whorl. (6) A subsutural band was added to the one described under 5.

Shells (eroded) had 5 ± 1 whorls. The average length and width were 25.6 and 9.1 mm, respectively. The average length of the body whorl was 15.2 mm. Where the body whorl length varied from 14.7 to 15.7 mm, the average length and width of the aperture were 9.9 and 5.6 mm, respectively, with a length/width ratio of 1.77.

Embryos (Pl. 8, Figs. 16-20)

Statistics on numbers of young per pallial brood pouch along with the percentages of young at each whorl stage are presented in Table 13; included are statistics on shell measurements. Data of use for performing analyses of variance of shell measurements are given in Appendix 1. Measurements of the apical whorl are given in Appendix 2.

Shells are glassy to opaque white; they are medium in size (Table 38) and elongate (Table 40). They typically have 2 pronounced spiral cords and are nodulate, not ribbed. The tip of the apex is not emergent but generally suppressed. The adapical cord appears at 1½ whorls on the shoulder of the whorl. Nodulation begins on this cord at 1¾ to 2 whorls and nodes average 15 on the first volution (Appendix 2). Looking down on the apex, the nodes are expanded laterally so that the cord has a scalloped appearance. On the shoulder of the whorl between the suture and the noded cord are 2 or 3 spiral threads or grooves (seen at 16× magnification). Only rarely does a node elongate into a low rib. The noded cord spirals anteriorly to form a distinct outpocketing on the outer lip about ⅓ the aperture height from the adapical tip (Pl. 8, Figs. 16-20).

In shells of 2½ whorls the abapical cord is evident only on the last half of the body whorl about 0.19 mm below the adapical cord. This also causes an outpocketing of the outer lip. In shells of 3 whorls, the 2 cords are very distinct, 0.24 - 0.31 mm apart. The abapical cord at this later whorl stage arises at the adapical tip of the aperture and spirals anteriorly (Pl. 8, Fig. 19). In 14% of the shells, a 3rd minor cord was seen between the 2 main cords; this cord was nodulate, the nodes being in line with those on the adapical cord. In a few cases the series of nodes was connected by a very low rib.

At a magnification of 16×, spiral threads or grooves are seen at mid-body whorl and near the base. These are crossed by fine growth lines. Under water and direct illumination, about 5 distinct spiral threads may be seen, looking through the aperture, on the basal portion of the body whorl, towards the outer lip. These will later become the pronounced basal cords in the adult.

A yellow or brown basal band was observed on 48% of the embryonic shells of 3 whorls; it was rarely seen on

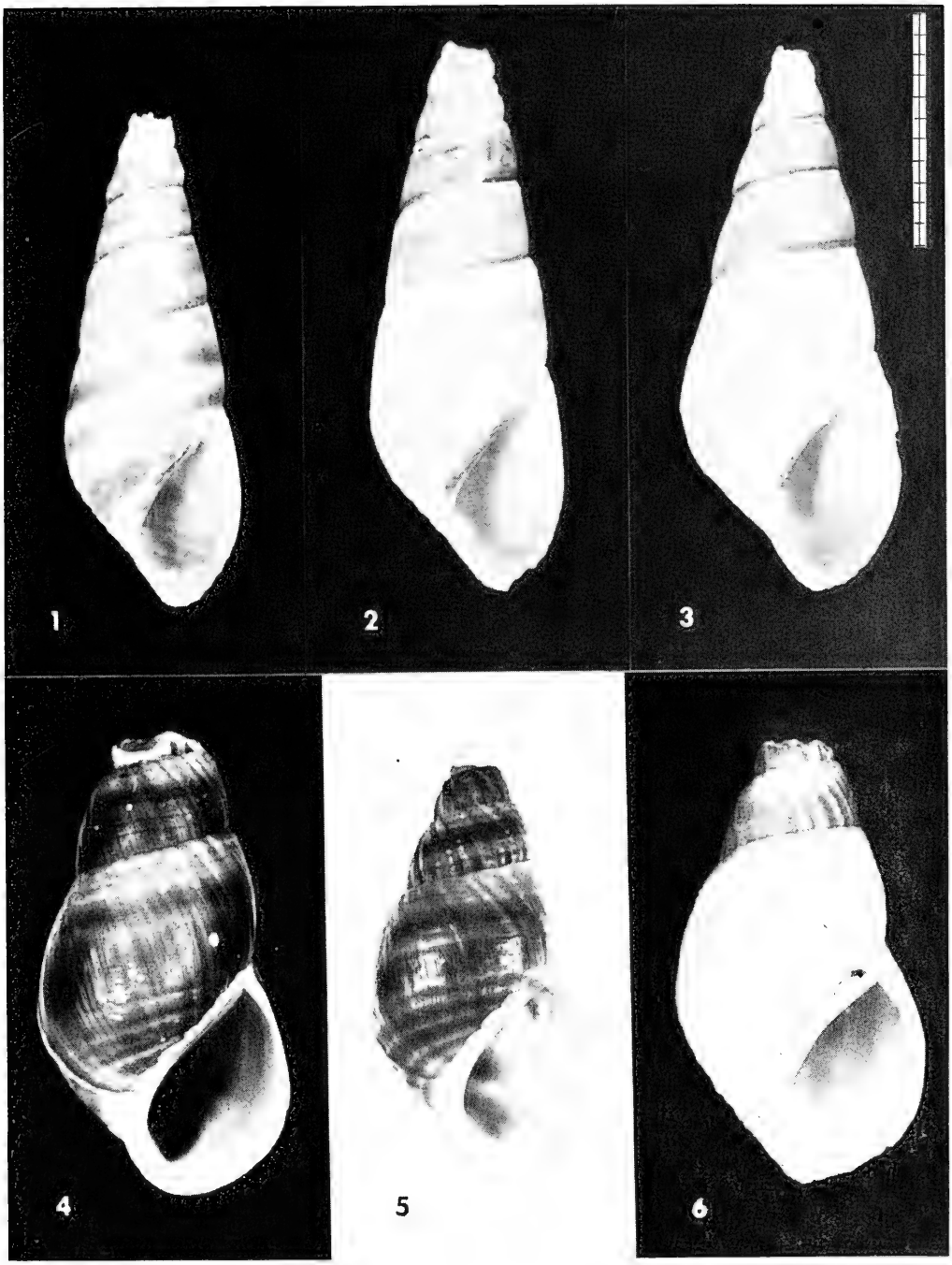


PLATE 2. Adult shells of 2 species of *Semisulcospira*. The measurement line is in mm.

FIGS. 1-3. *Semisulcospira kurodai* Kajiyama & Habe. Note how the ribs fade out on the apical whorls.

FIGS. 4-6. *Semisulcospira nakasekoae* Kuroda. The finely noded ribs of this species fade out on the penultimate whorl of fully mature specimens.

TABLE 13. Embryo shells; a statistical analysis of numbers per female and shell features of *Semisulcospira kurodai*

Number females examined	Number young per female	Feature studied	Mean measurements (mm) of embryos of different whorl counts				
			< 2.0	2.0	2.5	3.0	3.5
13	$\bar{X}$, 35.5	Shell length		0.96	1.25	1.62	--
	S, 15.4	Shell width		0.83	0.99	1.15	--
	Se, 4.27	Body whorl L.		0.81	1.02	1.23	--
		Ratio L/W		1.16	1.26	1.41	
	Range 18-66	% total embryos	60.6	20.3	13.1	6.0	0

$\bar{X}$ = mean
S = standard deviation
Se = standard error of the mean
L = length
W = width

TABLE 14. Adult shells; a statistical analysis of features of *Semisulcospira nakasekoae*

No. specimens examined	Statistic	Feature measured or counted					
		Spire angle	Ribs	Basal cords	Body whorl length	Shell length (mm)	Shell width (mm)
25	$\bar{X}$	29.1°	16.8	4.6	16.2	19.8	10.9
	S	4.10	1.07	0.86	1.07	--	0.85
	Se	0.82	0.38	0.17	0.21	--	0.17

$\bar{X}$ = mean
S = standard deviation
Se = standard error of the mean

TABLE 15. Color patterns of adult shells of *Semisulcospira nakasekoae*

Number specimens examined	Uniform yellow	Uniform brown to purple-black	Banded (normal)	Banded (irregular patterns)
111	13.5	10.8	66.6	9.1

shells of 2.5 whorls although many shells of the latter had a tinge of yellow at the columellar base.

Ontogeny of Sculpture

Ribs never develop on the shells of some post-embryonic young. In these cases, nodes are present at mid-whorl up to the 5th whorl, but fade out on the 6th. In another variation, the nodes at mid-whorl on the 4th whorl are actually the summits of low ribs. By the 5th whorl ribs are more pronounced, but still low, with one spiral series of nodes at mid-whorl and an adapical series close to the suture. In the 6th whorl the ribs are the most pronounced.

A 3rd class of variants is found where ribs are narrow, long pronounced folds from whorls 5 to 8. Nodes fade out by the 7th whorl and the ribs fade out as described for adult shells.

Comparison of species

Semisulcospira kurodai is a member of the *S. libertina* complex. The adult shell is different from those of other taxa when the ontogeny of ribs and spire angle are considered together. Ribs do not develop past the 6th to 8th whorl and are not present on the penultimate whorl of mature snails. The only other species with a somewhat similar ontogeny of ribs is *S. nakasekoae*. The former taxon has a spire angle of 16.6° ± 3.08° while that of the latter is 29.1° ± 4.10°. *S. kurodai* is separated from *S. libertina* by having significantly fewer basal cords (5.10 ± 1.02 as compared to 9.2 ± 1.6). The embryos of the former are white or glassy while those of the latter are brownish.

Semisulcospira nakasekoae
Kuroda, 1929

Station 8, Topotypes

Adults (Pl. 2, Figs. 4-6)

Statistics on basic shell features are given in Table 14. Shells were very sturdy, short and with a large spire angle (29.1° ± 4°). In over 60% of the population the adults had only 2 whorls. Ribs were observed and countable only on the most apical whorl in 32% of the population. The ribs faded out on the body-whorl and often on the penultimate whorl; they averaged 0.19 mm in diameter, were low, and frequently slanted from left to right (adapical to abapical). In some young specimens the ribs were nodulate (8 to 10 nodes) owing to cords passing over them. In some cases, nodulation was present in the absence of cords.

In 28% of the shells the mid-whorl was smooth. Cords often were depressed so that intercord grooves were more evident. When cords were pronounced, 8 to 10 were counted on the penultimate whorl.

Color patterns are presented in Table 15. In "uniformly yellow" shells the apical whorl was often a darker yellow-brown than the body whorl (Pl. 2, Fig. 6). In normal banded shells a yellow stripe at mid-whorl was flanked on either side by wide colored bands (purple-brown to mixtures of blue, green and brown). In many shells the bands appeared blue-gray. Bands faded out $\frac{1}{5}$ to $\frac{1}{2}$ whorl back from the aperture where the yellow shell background color of the lip narrowed to a U-shaped wedge between them. Irregular color patterns occurred when the bands were diffuse, indistinct or interrupted.

Shells of 2 or 3 whorls had an average length and width of 19.8 and 10.9 mm, respectively. The length of the body whorl averaged 16.2 mm. When the length of the body whorl varied from 15.7 to 16.7 mm, the average aperture length and width were 10.9 and 6.9 mm, respectively, with a length/width ratio of 1.58.

Embryos (Pl. 9, Figs. 1-5)

Statistics on numbers of young per pallial brood pouch and the percentages of young at each whorl stage are given in Table 16; included are statistics on shell measurements. More complete

TABLE 16. Embryo shells; a statistical analysis of numbers per female and shell features of *Semisulcospira nakasekoae*

Number females examined	Number young per female	Feature studied	Mean measurements (mm) of embryos of different whorl count				
			< 2.0	2.0	2.5	3.0	3.5
	$\bar{X}$, 19.7	Shell length		1.14	1.59	2.28	2.99
	S, 10.1	Shell width		1.05	1.38	1.86	2.40
17	Se, 2.4	Body whorl L.		1.05	1.42	2.01	2.57
		Ratio L/W		1.09	1.15	1.23	1.25
	Range 1-35	% total embryos	56.0	15.5	15.2	11.0	2.3

$\bar{X}$ = mean
S = standard deviation
Se = standard error of the mean
L = length
W = width

TABLE 17. Color patterns of *Semisulcospira nakasekoae* embryo shells

Embryo whorl stage	No. embryos examined	Color type (% of embryos)			
		(1) uniform light yellow	(2) uniform dark brown	(3) sutural band weak and basal band	(4) sutural band strong and wide basal band
2.0	25	100.0	0	0	0
2.5	21	14.2	4.8	81.0	0
3.0	24	8.3	0	41.7	50.0
3.5	9	11.1	0	11.1	77.8

TABLE 18. Shell measurements (mm) of the type series of *Semisulcospira habei*

Type series	Shell		Aperture		Body whorl length	Spire angle	Ribs
	Length	Width	L	W			
Type specimen	25.5	9.8	10.7	6.3	16.3	21°	13
Paratypes 1	23.3	9.5	9.8	5.5	14.5	22°	22
2	23.8	9.6	10.3	5.6	15.2	24°	23
3	24.5	9.2	10.0	5.2	15.4	18°	22
4	27.0	10.7	10.9	7.0	17.8	16°	12

L = length
W = width

data are given in Appendix 1.

Embryo shells are large (Table 38) and globose (Table 40). The apical whorl is flat with the tip emergent, level with the whorl or suppressed. Measurements of the apical whorl are given in Appendix 2. Ribs (Appendix 2) are present but not cords. Low ribs begin at 1¾ to 2 whorls and do not become pronounced until 2½ to 3 whorls; they are not nodulate. Five to 6 spiral grooves may be seen on the base of the shell. There are 4 color patterns (Table 17); the frequency of a particular pattern definitely changes as the whorl stages increase. The light yellow shell at 2.0 whorls changes at 2.5 whorls when a faint purple band starts at the suture and spirals abapically but does not continue out on the mid-body whorl. A 2nd purple-brown basal band is seen. At 3.0 whorls and later the sutural band continues out into the mid-body whorl, the intensity in band color increases, and both bands become wider. From 8 to 14% of the shells remain yellow (Tables 15, 17). A uniformly dark embryo shell is rare.

Comparison of species

This species is a member of the *Semisulcospira niponica* species complex. It is distinguished from other species by having an extremely great spire angle ($\bar{X}$, 29.1°; Text Fig. 18), ribs which fade out on the penultimate whorl of fully mature snails, and embryos which are globose at 3.5 whorls (at which time they are liberated from the pallial brood chamber).

Semisulcospira habei, new species

Station 8, Type Population

Type series (Pl. 3, Figs. 1-3)

The type specimen (Pl. 3, Fig. 1) is a female (UMMZ 220236) with a brood pouch that contained 31 young. There are 13 paratypes (UMMZ 220237), of which 4 were cleaned in Clorox. This series is deposited in the Museum of Zoology, University of Michigan. Statistics on the type and selected paratypes are given in Table 18. The description that follows is based on data from the type series plus 12 additional snails of the same population.

Adults

Statistics on prominent adult features are given in Table 19. Ribs are more prominant than spiral cords. They are distinctly nodulate, the nodes corresponding to the points where spiral cords pass over the ribs. In many specimens cords are not present; when cords are absent, intercord spiral grooves are often seen passing over the ribs. Generally, there are 6 ± 1 spiral cords. The 3 or 4 basal cords on the body whorl are all distinct.

As shown in Table 20, there are 2 color patterns. Dark colored shells are uniform blue to blue-purple. The body whorl may be purple. In dark shells, ribs stand out owing to their white nodes (on cleaned shells). Banding is masked by the dark background color in dark shells. The outer lip is white to yellow-white. The clear area narrows like the point of a V at mid-whorl and extends back along the whorl from the outer lip often as far as ½ whorl. This divides the dark shell into 2 bands on the body whorl near the outer lip.

Looking into the aperture at the inside wall of the outer lip (sometimes seen on the exterior body whorl at the base near the lip), it can be seen that the abapical dark band is frequently split into 3-5 brown bands, each following along one of the cords (Pl. 3, Figs. 2, 3).

Most shells had 4 whorls, but a few had 3 or 5. They averaged 25.3 and 10.5 mm in length and width, respectively. The length of the body whorl averaged 16.9 mm. Where the length of body whorl varied from 16.4 to 17.4 mm, respectively, the average length and width of the aperture were 10.7 and 6.2 mm, respectively, with a length/

TABLE 19. Adult shells; a statistical analysis of features of *Semisulcospira habei*

No. specimens examined	Statistic	Feature measured or counted					
		Spire angle	Ribs	Basal cords	Body whorl length	Shell length (mm)	Shell width (mm)
17	$\bar{X}$	18.0	20.8	4.0	16.9	25.3	10.5
	S	2.84	3.9	0.15	1.70	--	1.08
	Se	0.68	0.95	0.05	0.47	--	0.26

$\bar{X}$ = mean
S = standard deviation
Se = standard error of the mean

TABLE 20. Color patterns of adult and embryo shells of *Semisulcospira habei*

% of 30 adults		% of 200 embryos*		
		yellow	dark	banded
yellow	40	58	0	42
dark	60	18	0	82

* 100 embryos from yellow adults, 100 from dark adults.

TABLE 21. Embryo shells; a statistical analysis of numbers per female and shell features of *Semisulcospira habei*

Number females examined	Number young per female	Feature studied	Mean measurements (mm) of embryos of different whorl count				
			<2.0	2.0	2.5	3.0	3.5
13	$\bar{X}$, 25.2	Shell length		1.21	1.63	2.23	2.59
	S, 9.85	Shell width		1.11	1.44	1.79	2.00
	Se, 2.73	Body whorl L.		1.09	1.43	1.86	2.09
		Ratio L/W		1.09	1.13	1.25	1.30
	Range 5-41	% total embryos	18.0	24.8	30.1	17.2	9.8

$\bar{X}$ = mean
S = standard deviation
Se = standard error of the mean

width ratio of 1.73.

Embryos (Pl. 9, Figs. 6-10)

Statistics on numbers of young per pallial brood pouch and the percentages of young at each whorl stage are given in Table 21; included are statistics on shell measurements. More complete data are given in Appendix 1.

Embryo shells are sturdy, large (Table 38) and globose (Table 40). The columella has a distinctive twist at the end of the parietal wall. Abapical to this twist the columella is thickened and reflected. Apical whorl measurements are given in Appendix 2.

Ribs are prominent (Appendix 2). They begin at 1¼ whorls and are strongly nodulate or spinose on the whorl shoulder. In shells of 2½ whorls the shoulder is sharp. Beneath the spiny process on the shoulder, each rib decreases in height and generally disappears before reaching the abapical suture. In shells of 3 or more whorls, the whorls are less shouldered and more convex. On these later whorls each rib has 2 to 4 nodes, the height of each rib is 0.13 to 0.18 mm and their diameters average 0.23 mm. The greatest rib height and most pronounced node are just adapical to mid-whorl. On the body whorl, ribs fade out abruptly at mid-whorl as a node or on a spiral cord. The cord, when present, is smooth or nodulate.

The color pattern of the young appears to be influenced by the color pattern of the adult (Table 20). Embryos are either uniform yellow or banded. When bands are extremely pronounced the shells take on a purple-red shade. Some white-yellow shells have a slight purple-brown tinge to the columella and shell next to the columella.

There are 3 bands in the majority of the banded shells (Pl. 9; Figs. 9, 10): a distinct purple-brown band at the suture (0.12 mm wide); one crossing the body whorl over the ends of the ribs (or cord when it is present); and an extremely wide basal band. In some rare cases the band at mid-whorl is very faint or missing.

Discussion of the species

The low number and large size of the embryos in each pallial brood pouch and the low number of basal cords on the adult shell separate this taxon from *Semisulcospira libertina* and *S. reiniana*.

Semisulcospira habei has pronounced nodulate ribs on the penultimate whorl of the strong adult shell and embryos. *S. nakasekoae* has a shorter and stouter shell, and ribs are not pronounced on the adult body whorl or embryos. *S. kurodai* is fragile and smooth, and its embryos are without colored bands and ribs (they only have nodes). One of the morphs of *S. multigranosa* is smooth and more slender.

Semisulcospira habei adult shells have 16 to 25 ribs on the penultimate whorl (68% of the population) and each rib has 5 to 7 nodes. *S. niponica* has only 10 to 12 ribs (68% of the population), with each bearing only 3 to 4 large pustulate nodes.

Semisulcospira habei is separated from *S. reticulata* and *S. multigranosa* by a number of fundamental differences. The latter taxa have 2 to 8 embryos per brood pouch (68% of the population), while the former has 15 to 35 (68% of the population). As seen in Table 38, *S. reticulata* has much larger embryos. Adult shells of *S. reticulata* have more ribs on the penultimate whorl than *S. habei* (26 or more in 68% of the former, while 25 or less in 68% of the latter; significant difference, $P = <0.01$). Embryos of *S. multigranosa* are elongate, while those of *S. habei* are globose (Table 40). The ribbed morph of *S. multigranosa* is significantly more slender and has more ribs ($P=0.01$) than *S. habei*.

Semisulcospira habei has a mean spire angle of 18° and *S. decipiens* has a mean spire angle of 12.4° (significant difference, $P=0.01$). The former has globose embryos where the majority are banded and the latter has elongate yel-

low-white embryos. The embryos of *S. habei* are larger than those of *S. decipiens* (Table 38).

Semisulcospira habei is contrasted to *S. habei yamaguchi* under that subspecies (see below).

Semisulcospira habei yamaguchi, new subspecies

Station 3, Type Population; Stations 6 and 7

Type series.

The type specimen is a female (Pl. 3; Fig. 4). The shell was broken, as were the shells of the entire type series, to obtain the gonad for cytological studies. The shell, prior to breaking, consisted of 4 whorls and measured 27.5 mm long and 9.5 mm wide. The apex was eroded. There are 23 ribs on the penultimate whorl with 6 nodes per rib. The spire angle is 14°. The type series, comprising the type (UMMZ 228801) and 4 paratypes (UMMZ 228802), is deposited in the Museum of Zoology, University of Michigan. The description that follows is based on the type series and 6 additional specimens from Stations 6 and 7.

Adults (Pl. 3; Figs. 4-6)

Statistics on fundamental shell features are given in Table 22. The 2-3 basal cords of the spiral cords on the body whorl are prominent, shells are slender, and each rib on the penultimate whorl has 5 to 6 pronounced nodes. As shown in Table 23, shells are a uniform yellow or banded. Banded shells have 2 patterns: (1) a sutural band spirals out onto the mid-body whorl, (2) the same as 1, but with an additional basal band and a wide, dark sub-sutural band.

Shells had 5 ± 1 whorls with an average length and width of 27.1 and 8.7 mm, respectively. The average length of the body whorl was 13.9 mm. The whorls are slightly convex. In older adults, sculpture on the body whorl is faint or absent.

Embryos (Pl. 9, Figs. 11-15)

Statistics on numbers of young per pallial brood pouch and the percentages of young at each whorl stage are given in Table 24; included are data on shell measurements. More complete statistics on shell measurements are given in Appendix 1.

Embryo shells were found in only 3 of the 11 available snails. Shells were medium in size (Table 38) and intermediate in shape (Table 40); they were fragile. Measurements of the apical whorl are given in Appendix 2. There were spinose ribs at the whorl shoulders which began at the 1¼ to 1¾ whorl stage. There were 14 ± 1 spinose nodes on the first volution (Appendix 2); when ribs were present they faded out by mid-whorl. Several minute spiral threads or grooves passed over the ribs.

Shells were uniform glassy to white or banded (Table 23). Banding started with a sutural brownish-purple band at 2 to 2½ whorls, which spiraled anteriorly to form a distinct band at mid-body whorl. There was also an accompanying basal band appressed to the columella.

Discussion of the species

This taxon was initially confused with both *Semisulcospira decipiens* and *S. multigranosa*, with which it is sympatric. It was first distinguished when it was found that snails appearing to be *S. decipiens* had the same low chromosome number as *S. habei* (2n=17 to 20 contrasted with 2n=25 or 26 for *S. decipiens* [Burch & Davis, 1967; Burch, 1968]).

The embryos of this taxon and *Semisulcospira decipiens* were quite different than those of *S. multigranosa*. When one compares the embryos of *S. habei yamaguchi* (Pl. 9; Figs. 11-15), *S. decipiens* (Pl. 10; Figs. 6-9) and *S. multigranosa* (Pl. 11; Figs. 5-8), it is evident those of *S. multigranosa* are

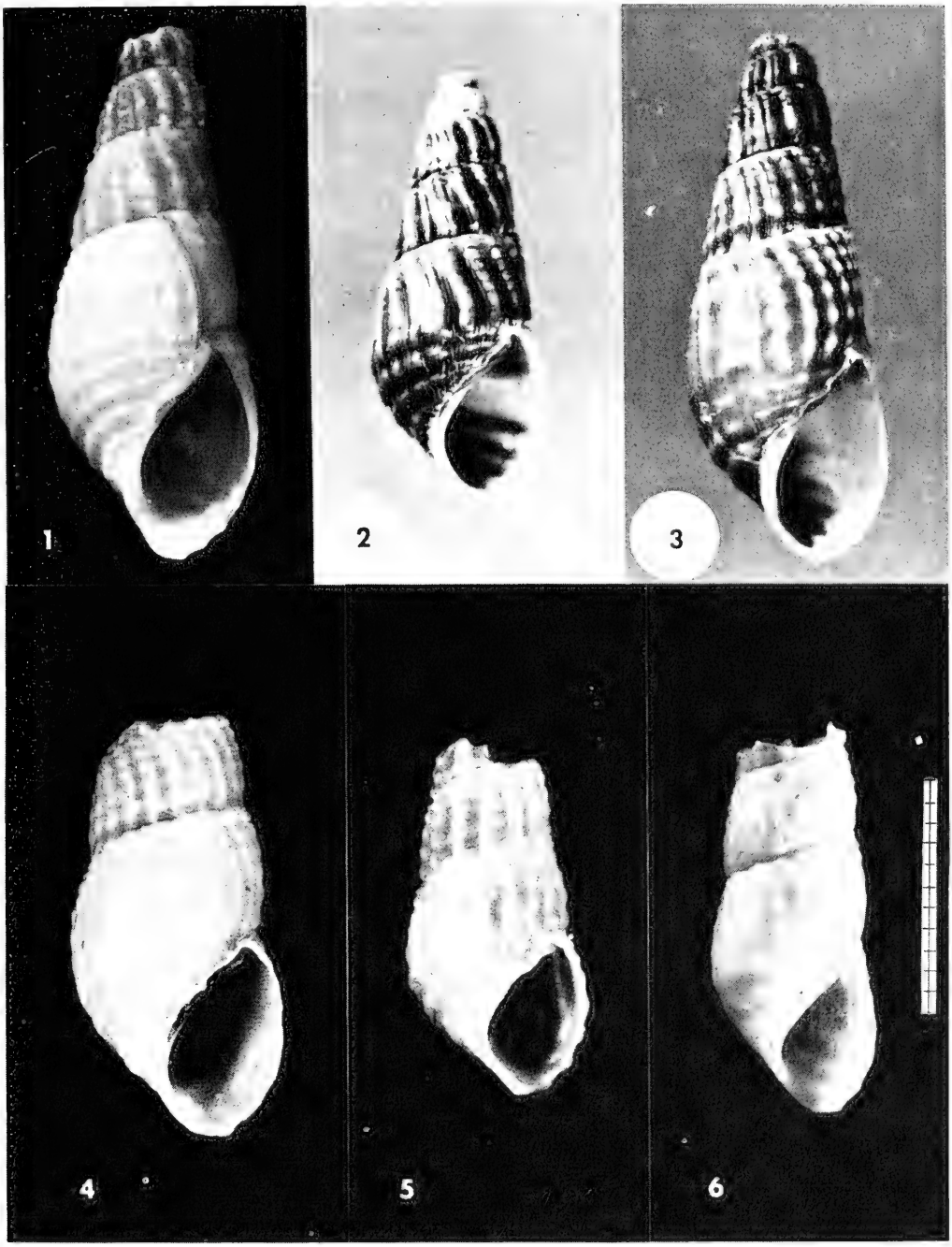


PLATE 3. Adult shells of 2 new taxa of *Semisulcospira*. Measurement line is in mm.

FIGS. 1-3. *Semisulcospira habei* new species. Fig. 1, Holotype; Figs. 2-3, Paratypes.

FIGS. 4-6. *Semisulcospira habei yamaguchi* new subspecies. Fig. 4, Holotype; Figs. 5-6, Paratypes. Apical whorls were removed to gain access to the gonad for cytological studies (Burch & Davis, 1967).

TABLE 22. Adult shells; a statistical analysis of features of *Semisulcospira habei yamaguchi*

No. specimens examined	Statistic	Feature measured or counted					
		Spire angle	Ribs	Basal cords	Body whorl length (mm)	Shell length (mm)	Shell width (mm)
11	$\bar{X}$	14.5°	18.0	2-3	13.9	27.1	8.7
	S	2.25	3.14	--	1.58	--	0.87
	Se	0.71	0.94	--	0.52	--	0.27

$\bar{X}$ = mean
S = standard deviation
Se = standard error of the mean

TABLE 23. Color patterns of adult and embryo shells of *Semisulcospira habei yamaguchi*

Adults (%)			Embryo (%)		
No.	Uniform yellow	Banded	No.	Uniform glassy-white	Banded
11	64	36	50	74	26

TABLE 24. Embryo shells; a statistical analysis of numbers per female and shell features of *Semisulcospira habei yamaguchi*

Number females examined	Number young per female	Feature studied	Mean measurements (mm) of embryos of different whorl count				
			< 2.0	2.0	2.5	3.0	3.5
3	$\bar{X}$, 17 ± 3	Shell length		1.06	1.35	1.78	2.15
		Shell width		0.96	1.15	1.35	1.56
		Body whorl L.		0.96	1.16	1.47	1.68
		Ratio L/W		1.10	1.17	1.32	1.38
		% total embryos	36.5	26.9	11.15	11.5	13.5

$\bar{X}$ = mean
L = length
W = width

much larger and more elongate (Tables 38, 40).

Adult shell features of *Semisulcospira habei yamaguchi* appear to overlap those of *S. decipiens*. However, the former has an average spire angle of 14.5° and the latter an average spire angle of 12.4° (significant difference, P=0.01 level). *S. habei yamaguchi* has an average of 18 ribs on the penultimate whorl, while *S. decipiens* has an average of 16 (not significantly different, P=0.10 level). The ribs of *S. decipiens* are, however, smooth to weakly nodulate with 7 to 8 nodes per rib, while ribs of *S. habei yamaguchi* are heavily nodulate with 5 to 6 nodes per rib.

Embryos of *Semisulcospira decipiens* and this taxon appear quite similar in size and structure. There are 2 to 3 significant differences. *S. habei yamaguchi* has a wider shell, as shown by the length/width ratios for different whorl stages (Table 40, Text Figs. 9, 10). *S. h. yamaguchi* has more ribs on the first volution of the embryonic shell (13 to 15 as compared to 10 to 12 for *S. decipiens*). Not only are the ribs more numerous, but they begin earlier on the embryonic shell (1¼ to 1¾ whorl as compared to 1¾ to 2 whorls on *S. decipiens*). They are also sharp, prominent and spinose as contrasted to the flatter, less prominent ribs of *S. decipiens*.

Until more specimens are made available for study, I am reluctant to designate this taxon as a full species. It is closely allied to *Semisulcospira habei habei* on the basis of chromosome number (2n=17 to 20) and by being present in the same drainage system (allopatric). Certain adult features appear to overlap. The spire angle of *S. h. habei* varies from 15° to 21° in 68% of the population, while that of *S. h. yamaguchi* varies from 12° to 17° in 68% of the population. However, the spire angles in the 2 populations are very significantly different (P=0.01 level). *S. h. habei* has significantly more ribs (P only at .05 level). Both taxa have 5 to 6 nodes per rib.

The taxa differ in that the embryonic shells of *Semisulcospira h. habei* are globose while those of *S. h. yamaguchi* are intermediate (Table 40). The embryos of the former are larger than those of the latter (Table 38). The columella of the latter's embryonic shell is not twisted as in the former, nor do the shells have the pronounced heavy banding peculiar to *S. h. habei*. No adult shells of *S. h. yamaguchi* had the purple or dark blue-purple shells seen in 60% of adult *S. habei*.

Semisulcospira niponica (Smith, 1876)

Station 1, Topotypes

Adults (Pl. 4; Figs. 1-3)

Statistics on basic shell features are presented in Table 25. The extremely sturdy shell has 2 basal cords on the body whorl in 92% of the population studied (77 specimens). There are 3-4 very large pustulate nodes on each rib of the penultimate whorl. Spiral cords are not in evidence except towards the outer lip, where one can count 6-8 faint cords for the body whorl.

As shown in Table 26, there were no light colored shells. In the mixed category, the apical whorls were often purple-black and the body whorl brown. Banding was not prominent or clearly defined because of the heavily noded ribs and generally dark shell. Banding was most pronounced on shells in the mixed category. Three dark brown bands (sutural, mid-whorl and basal) crossed the body whorl when banding was evident. The sutural bands on the penultimate whorl were divided by a yellow central band. Looking into the aperture, brownish bands on the inside of the outer lip following the basal cords, and 1 or 2 brownish-purple bands toward the suture (Pl. 4, Fig. 2) frequently could be observed.

Adult shells were eroded at the apex, had 3 or 4 whorls and averaged 22.8 and 10.1 mm in height and width, respec-

TABLE 25. Adult shells; a statistical analysis of features of *Semisulcospira niponica*

No. specimens examined	Statistic	Feature measured or counted					
		Spire angle	Ribs	Basal cords	Body whorl length (mm)	Shell length (mm)	Shell width (mm)
25	$\bar{X}$	20.0	11.4	2-3	15.4	22.8	10.1
	S	2.80	1.23	--	0.80	--	0.50
	Se	0.56	0.25	--	0.16	--	0.10

$\bar{X}$ = mean
S = standard deviation
Se = standard error of the mean

TABLE 26. Color patterns of adult and embryo shells of *Semisulcospira niponica*

Adults (%)				Embryos (%)		
No.	Light-brown	Purple-black	Mixed	No.	Unbanded	3 bands
77	12.9	38.9	48.2	300	5	95

TABLE 27. Embryo shells; a statistical analysis of numbers per female and shell features of *Semisulcospira niponica*

Number females examined	Number young per female	Feature studied	Mean measurements (mm) of embryos of different whorl count				
			<2.0	2.0	2.5	3.0	3.5
14	$\bar{X}$, 21.6	Shell length		1.14	1.59	2.35	2.83
	S, 11.68	Shell width		1.08	1.44	1.87	2.11
	Se, 3.12	Body whorl L.		1.04	1.42	2.01	2.30
		Ratio L/W		1.06	1.10	1.26	1.34
	Range 2-49	% total embryos	38.3	21.4	17.5	13.5	9.2

$\bar{X}$ = mean
S = standard deviation
Se = standard error of the mean
L = length
W = width

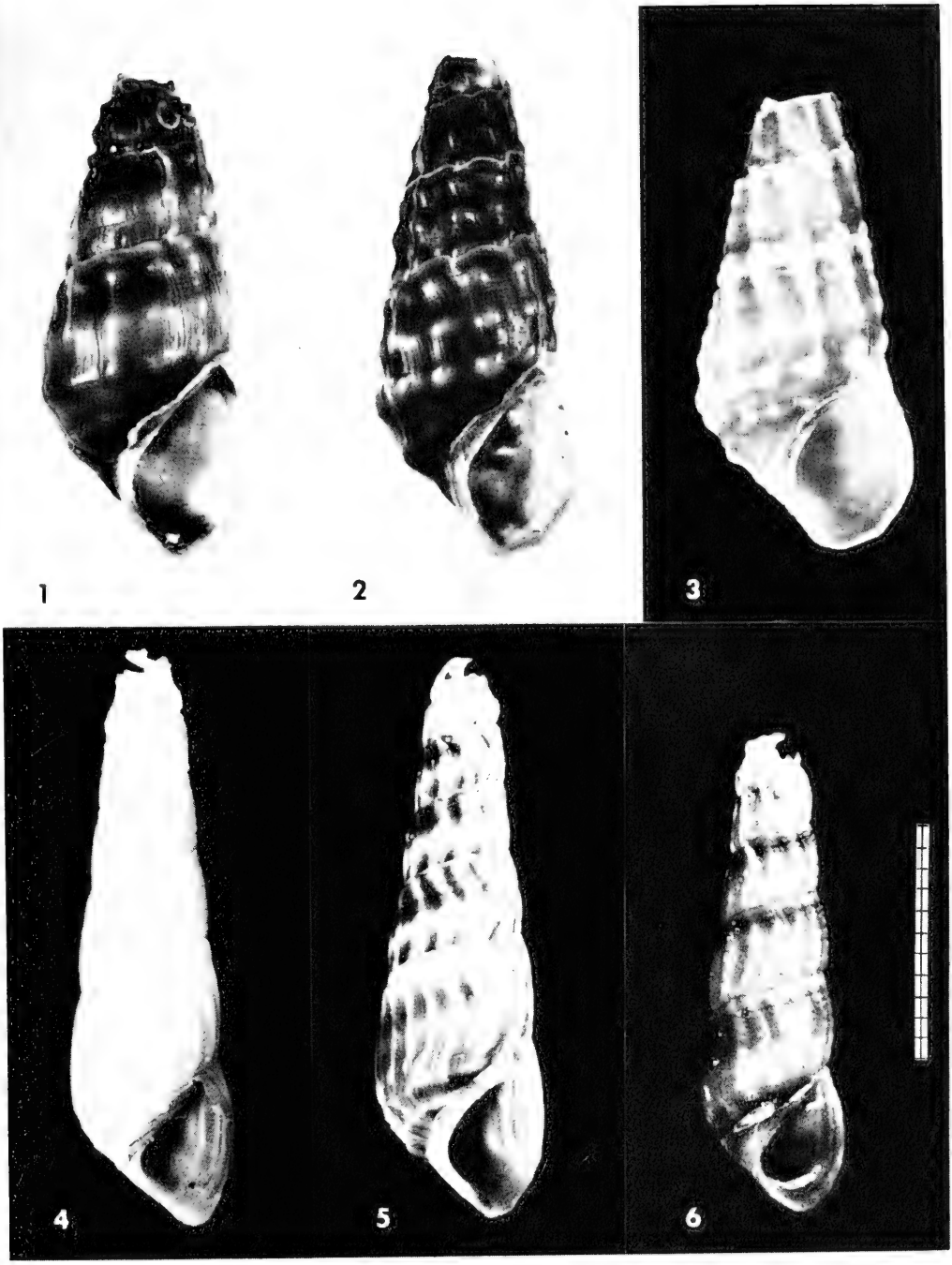


PLATE 4. Adult shells of 2 species of *Semisulcospira*. Measurement line in mm.

FIGS. 1-3. *Semisulcospira niponica* (Smith).

FIGS. 4-6. *Semisulcospira decipiens* (Westerlund).

tively. When the length of the body whorl varied from 14.9 to 16.9 mm, the average length and width of the aperature were 9.7 and 6.2 mm, respectively, with a length/width ratio of 1.56.

Embryos (Pl. 10, Figs. 1-5)

Statistics on numbers of young per pallial brood pouch and the percentages of young at each whorl stage are given in Table 27; included are data on shell measurements. More complete statistics on shell measurements are given in Appendix 1.

The sturdy embryo shells are large (Table 38) and globose (Table 40). They are characterized by nodulate ribs (Appendix 2), which first appear at 1¼ to 1½ whorls as nodes. The nodes become elongate at the 2nd whorl. The height of the ribs decreases rapidly toward the abapical whorl. In later whorls the position of the nodes spirals anteriorly from the shoulder to a mid-whorl position. In shells of 3 or more whorls, the rib is most swollen transversely at the mid-whorl region, where it has a diameter of 0.24 mm. Ribs terminate on a low cord.

The outer lip of shells with 2 to 2½ whorls is flared out and especially bent outward where the cord reaches the lip. This outfolding occurs about 1/3 the distance from the adapical tip of the aperture. Where shells have 3½ or more whorls the outfolding becomes less distinct and is often absent. Looking down on the shoulder of the apical whorls, spiral grooves can be observed on the shoulder of the whorls and running between the ribs.

Shells of 2½ whorls and larger have 3 distinct purple-brown bands (95%, Table 26); these are sutural, a slight one at mid-whorl, and a pronounced basal band. The sutural band shows up at the beginning of the 2nd whorl. The 2 apical whorls are light brown. In later whorls the shell background is white to yellow-white. In a few cases, the background is brown with the mid-whorl band especially pronounced (often 0.36 mm wide at 2½ whorls). The bands were a solid purple-black in 5% of the shells, but 4% of the embryos were from 1 female (50% from that female were dark; the female is suspected to have been solid purple-black).

Comparison of species

This species is a member of the *Semisulcospira niponica* species group. The species is characterized by having a blackish shell, on which each rib has 3 to 4 large pustulate nodes (contrasted with conditions in all other taxa). While some *S. multigranosa* and *S. decipiens* have blackish shells, the shells are either smooth (one morph of the former taxon) or the ribs have more than 6 nodes per rib. *S. niponica* has fewer ribs on the penultimate whorl than other taxa in the complex (Text Fig. 13).

Semisulcospira decipiens
(Westerlund, 1883)

Station 3, Topotypes

Adults (Pl. 4; Figs. 4-6)

Statistics on basic shell features are given in Table 28. Shells are characteristically ribbed, the ribs tending to curve with the outer lip. The ribs terminate on the body whorl on a cord which spirals from the penultimate whorl onto the body whorl at a level of the adapical tip of the aperture. This cord is either smooth or nodulate. In 11% of the population the ribs are smooth and are not crossed by spiral grooves; in 89% the ribs appear slightly nodulate, with 7 or 8 nodes per rib.

In 20% of all shells examined, spiral grooves crossed the ribs, but not the inter-rib shell surface. In 69% the grooves passed across both ribs and inter-rib shell. The grooving over the ribs causes the ribs to appear nodulate. Cords appear to be lateral expansions of the nodes on the ribs. These are, on any whorl, irregularly positioned, i.e., a cord may not complete a whole volution, or all nodes on a rib may not

TABLE 28. Adult shells; a statistical analysis of features of *Semisulcospira decipiens*

No. specimens examined	Statistic	Feature measured or counted					
		Spire angle	Ribs	Basal cords	Body whorl length (mm)	Shell length (mm)	Shell width (mm)
20	$\bar{X}$	12.4	16.0	2-4	13.8	26.3	8.30
	S	1.42	3.00	--	0.73	--	0.59
	Se	0.32	0.67	--	0.16	--	0.13

$\bar{X}$ = mean
S = standard deviation
Se = standard error of the mean

TABLE 29. Color patterns of adult and embryo shells of *Semisulcospira decipiens*

389 Adults (%)		173 Young* (%)		
		Uniform yellow	Uniform black	Banded
Uniform yellow	84.0	92.0	0	8.2
Uniform purple-black	3.0	43.5	0	56.5
Banded	13.0	60.0	0	40.0

* analyzed by correlation with adult color

TABLE 30. Embryo shells; a statistical analysis of numbers per female and shell features of *Semisulcospira decipiens*

Number females examined	Number young per female	Feature studied	Mean measurements (mm) of embryos of different whorl count					
			< 2.0	2.0	2.5	3.0	3.5	4.0
31	$\bar{X}$, 13.8	Shell length	--	--	1.35	1.83	2.23	--
	S, 7.67	Shell width	--	--	1.08	1.28	1.42	--
	Se, 1.38	Body whorl L.	--	--	1.30	1.53	1.85	--
		Ratio L/W	--	--	1.25	1.43	1.54	--
	Range 2-33	% total embryos	[(2.5) 32.1]		37.1	17.5	11.6	1.6

$\bar{X}$ = mean
S = standard deviation
Se = standard error of the mean
L = length
W = width

have lateral expansions into cords.

Shell color patterns are given in Table 29. In the category of uniform yellow, a few specimens varied from light yellow through brownish-yellow to uniform light brown. Such brown shells without banding are rare (less than 1%). Banded shells have a yellow stripe at mid-whorl flanked on either side by purplish-brown bands. The basal band on the penultimate whorl is closely appressed to the abapical suture; it continues onto the body whorl as a single band which expands to the shell's base. Variation in banding results from the varying degree in the width of each band. In some cases the central yellow stripe is very wide; in others, this spiral band is almost obliterated by the very wide flanking bands, with the result that the shell appears black. The extreme condition is a totally purple-black shell resulting from fusion of bands.

The eroded adult shells had 5 ± 1 whorls. When the length of the body whorl varied from 13.1 to 14.1 mm, the average length and width of the aperture were 8.5 and 5.0 mm, respectively, with a resulting length/width ratio of 1.70.

Embryos (Pl. 10, Figs. 6-9)

Statistics on numbers of young per pallial brood pouch and the percentage of young at each whorl stage are given in Table 30; included are data on shell measurements. More complete statistics on shell measurements are given in Appendix 1. Measurements of the apical whorls are given in Appendix 2.

The shells are characteristically medium in size (Table 38), elongate (Table 40) and fragile. Ribs are present, appearing first as low nodes on the shoulder at 1¾ whorls (Appendix 2). The nodes become elongated into ribs at the 2nd whorl. In shells of 2½ to 4 whorls the ribs do not pass across the periphery as a bar of uniform height; they are most pronounced at the shoulder where they are swollen but not spinose. Just below mid-whorl the ribs are interrupted by a distinct spiral groove 0.05 to 0.07 mm wide. The height of the rib decreases markedly, or is absent below the groove (abapically). In a few shells, ribs below the spiral groove are nodulate, the nodes expanding laterally into a spiral cord. This last feature is more characteristic of adult shells. Spiral grooves are seen at 6 to 16× magnifications crossing the ribs and inter-rib shell.

The percentage of young of a given color pattern depends upon the pattern in the parent (Table 29). The darker the adult shell (banded to uniform purple-black) the greater the frequency of banded young. Banding is variable: (1) some shells have only a brownish basal band, or (2) some shells have basal and mid-whorl bands, the latter circling just abapical to the point where the ribs terminate. Some shells are uniformly brown with a darker purple-brown basal band. Where young are obtained from purple-black adults, 61% have a decidedly brown shell.

Comparison of species

Semisulcospira decipiens is a member of the *S. niponica* species group. Because of the slender shell (spire angle $12.4° \pm 1.42°$) and ribs, it may be confused with *S. multigranosa* and *S. habei yamaguchi*. It differs from the former by having fewer ribs (16.0 ± 3.0 as compared to 23.2 ± 2.4) and smaller embryos (medium size against large; Table 38, p 260). The nature of the ribs on the embryos also is different (Compare Pl. 10, Figs. 6-9 to Pl. 11, Figs. 5-8). Both taxa are polymorphic in color pattern; however, no embryos of *S. decipiens* have been found which are uniform black, which is not the case for *S. multigranosa*. *S. decipiens* does not have smooth morphs as does *S. multigranosa*.

Semisulcospira decipiens was contrasted with *S. habei yamaguchi* in the section on the latter taxon.

Semisulcospira reticulata Kajiyama and Habe, 1961

Station 5, Topotypes; Stations 2 and 4

The species concept for *Semisulcospira reticulata* described here was derived from 3 different populations. Too few specimens were collected at the type locality (Station 5) to permit adequate analysis of shell or embryos. Populations of a size adequate for analysis were sampled at stations 2 and 4. Two different growth forms were found at station 4, the differences of which are discussed below.

Adults (Pl. 5, Figs. 1 and 2; Pl. 6, Figs. 1 and 2)

There are 2 different growth forms. Type 1 is identified by shells with moderately convex whorls, where the body whorl swells noticeably outside the angle formed by preceding whorls (Pl. 6, Fig. 1). The body whorl at the suture often appears crimped inwardly, giving the shell outline an angular to undulate appearance. In this crimped area, 2 to 4 spiral cords may be rather pronounced. The body whorl often appears lighter in color, and in older specimens advanced age is shown by the lack of clearly defined sculpture.

Type 2 growth form (Pl. 6, Fig. 2) closely resembles the figured type (Kajiyama & Habe, 1961). The whorls are flat sided with only the body whorl showing a slight convexity in some cases. Sculptured ribs are pronounced on the body whorl nearly the same length as that of type 1 shells where clearly defined sculpture is lacking.

Statistics on fundamental shell features are given in Table 31. Shells of both types are extremely sturdy. Ribs are numerous, pronounced and highly nodulate, with 6 ± 1 nodes per rib. The nodes correspond to cords, which vary in prominence from pronounced to absent.

Adult color patterns are given in Table 32. Banded shells had a purplish-brown band at mid-whorl (Pl. 6, Fig. 2). The band generally fades out or is missing on the body whorl. In some older specimens, the band is missing on the penultimate whorl. A basal band is present in some younger specimens (length of body whorl, 13.5 mm).

Shells from station 2 were all Type 1; they had 4.5 ± 1 whorls. With the length of the body whorl averaging 17.0 mm, the average length and width of the aperture were 10.5 and 6.8 mm, respectively, with a length/width ratio of 1.54. Collections at station 4 were made on 24 July and 10 November, 1965. In the former collection shells were recovered from shell mounds and were sub-fossils; 76% were type 2 and 24% were type 1. These sub-fossils were considerably larger than those from station 2. Those of shell type 2 averaged 7.5 whorls. When the body whorl length varied from 17.8 to 18.8 mm, the average length and width of the aperture were 11.5 and 7.9 mm, respectively, with a length/width ratio of 1.46. Those of shell type 1 had 6 whorls. When the body length varied from 21 to 22 mm, the average length and width of the aperture were 13.5 and 4.0 mm, respectively, with a length/width ratio of 1.50.

Living material was collected from the bottoms of fishing boats at station 4 on 10 November, 1965. These were type 1 only and had 5.5 ± 1 whorls. When the length of the body whorl ranged from 18.2 to 19.2 mm, the average length and width of the aperture were 12.2 and 7.4 mm, respectively, with a length/width ratio of 1.65.

Embryos (Pl. 10, Figs. 10, 11; Pl. 11, Figs. 1-4)

Embryos from parental stock collected at station 2 were studied in detail. Statistics on numbers of young per pallial brood pouch and the percentages of young at each whorl stage are given in Table 33; included are data on shell measurements. More complete statistics on shell measurements are given in Ap-

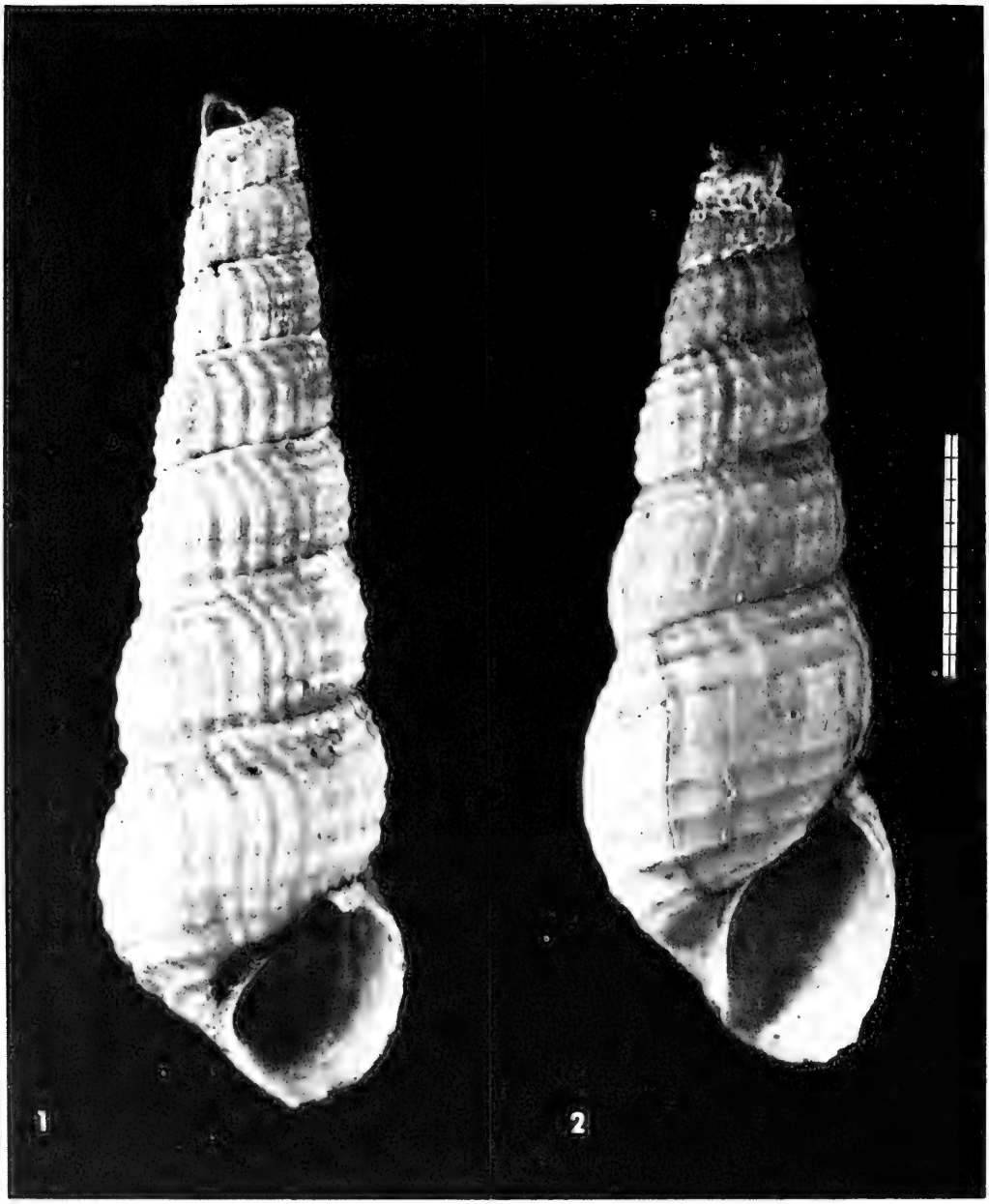


PLATE 5. Adult shells of *Semisulcospira reticulata* Kajiyama & Habe. The measurement line is in mm.

FIG. 1. Shell type 2 conforms to the "type."

FIG. 2. Shell type 2. The specimen shown is larger than that in Fig. 1 as determined by the greater length of the body whorl. Irregularity of sculpture on the body whorl indicates advanced age, as does the increased convexity of the body whorl relative to the most apical 4 whorls.

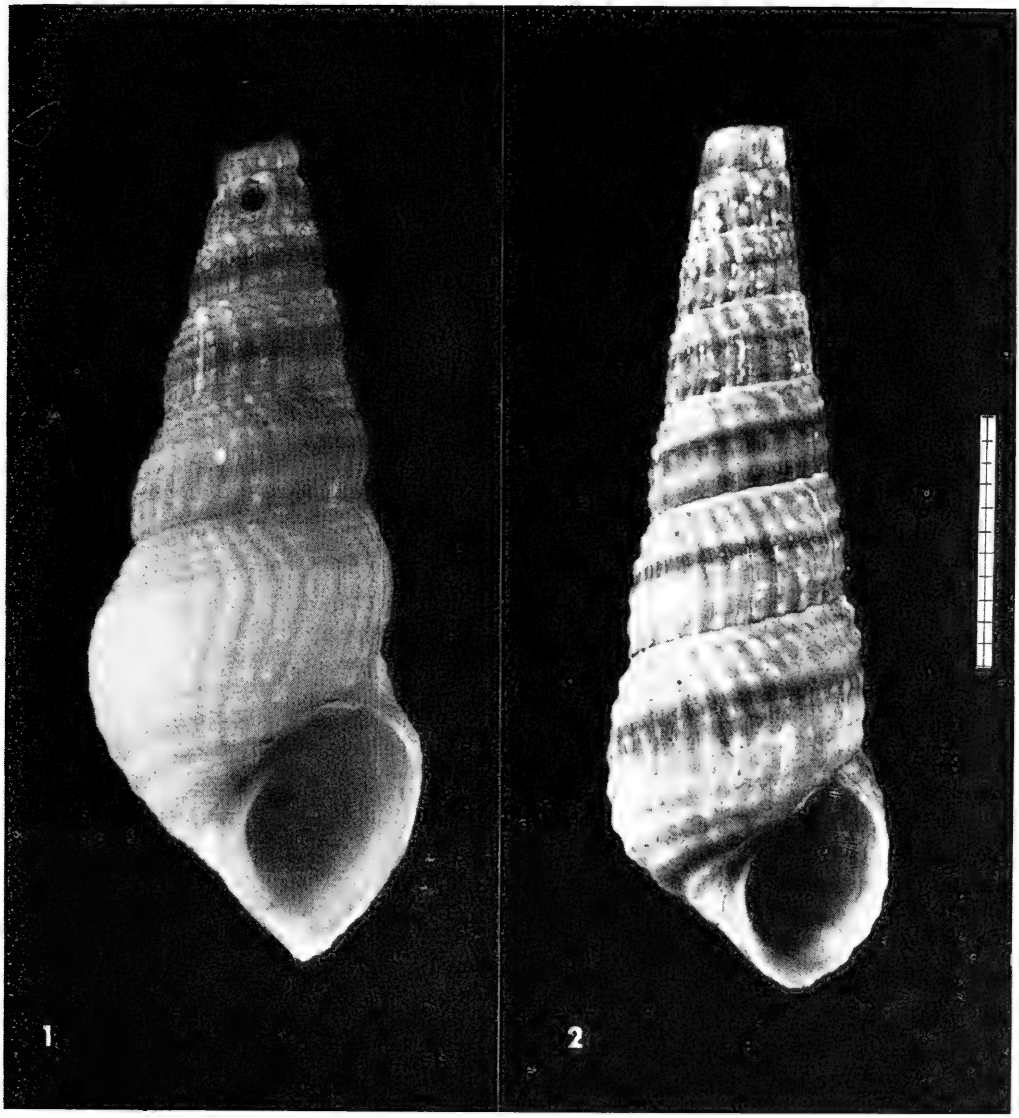


PLATE 6. Adult shells of *Semisulcospira reticulata* Kajiyama & Habe. The measurement line is in mm.

FIG. 1. Shell type 1. Note the convex whorls starting with the 4th whorl from the apex. The body whorl is markedly convex, yet the sculptural pattern is clear and regular.

FIG. 2. Shell type 2 conforms to the "type." Note the flatsided whorls.

TABLE 31. Adult shells; a statistical analysis of features of *Semisulcospira reticulata* collected from 2 populations

Station (Collecting site)	Statistical measurement	Feature measured or counted								
		Spire angle	Ribs	Basal cords	Nodes on ribs	Body whorl length (mm)	No. of whorls	Shell length (mm)	Shell width (mm)	No. specimens examined
Site 2	$\bar{X}$	17.3°	31.0	3-4	6.6	17.0	4.5	30.2	11.8	
Living shell	S	3.35	3.70	-	0.83	1.3	±1.0	-	1.15	36
type 1	Se	0.67	0.65	-	0.13	0.21	-	-	0.19	
Site 4	$\bar{X}$	16.4°	29.1	3-4	6.0	21.5	6.0	42.7	14.9	
Sub-fossil shell	S	1.15	2.48	-	0.65	1.18	0.0	-	0.94	8
type 1 (July 24)	Se	0.41	0.94	-	0.23	0.42	0.0	-	0.33	
	$\bar{X}$	15.8°	29.0	3-4	6.0	18.3	7.5	43.0	13.6	
Shell type 2	S	1.52	3.00	-	0.68	1.0	0.96	-	0.79	20
	Se	0.34	0.67	-	0.15	0.22	0.21	-	0.18	
Site 4	$\bar{X}$	18.1°	30.5	3-4	6.2	18.7	5.5	35.3	12.9	
Living shell	S	3.29	5.48	-	0.98	1.06	0.80	-	0.77	15
type 1 (Nov. 10)	Se	0.85	1.56	-	0.27	0.27	0.22	-	0.20	

$\bar{X}$ = mean
S = standard deviation
Se = standard error of the mean

TABLE 32. Color patterns of adult and embryo shells of *Semisulcospira reticulata*

%	Population	No. of specimens	Uniform yellow or brown	Banded
Adults	Site 2 Living shell type 1	428	74	26
	Site 4 Living shell type 1	19	63	37
	Site 4 Sub-fossil shell type 1	28	54	46
	Site 4 Sub-fossil shell type 2	115	55	45
Embryos	Site 2 From living adults of shell type 1	100	48	52

TABLE 33. Embryo shells; a statistical analysis of numbers per female and shell features of *Semisulcospira reticulata* from population 2 with adults of shell type 1

Number females examined	Number young per female	Feature studied	Mean measurements (mm) of embryos of different whorl count							
			<2.0	2.0	2.5	3.0	3.5	4.0	4.5	5.0
21	$\bar{X}$, 4.8	Shell length		1.24	1.82	2.59	3.60	4.71	5.65	-
	S, 3.40	Shell width		1.19	1.64	2.07	2.61	3.12	3.57	-
	Se, 0.74	Body whorl L.		1.18	1.59	2.24	2.98	3.64	4.27	-
		Ratio L/W		1.04	1.11	1.25	1.38	1.51	1.58	-
	Range 1-13	% total embryos	20.0	14.0	24.0	12.0	17.0	7.0	4.0	2.0

$\bar{X}$ = mean
S = standard deviation
Se = standard error of the mean
L = length
W = width

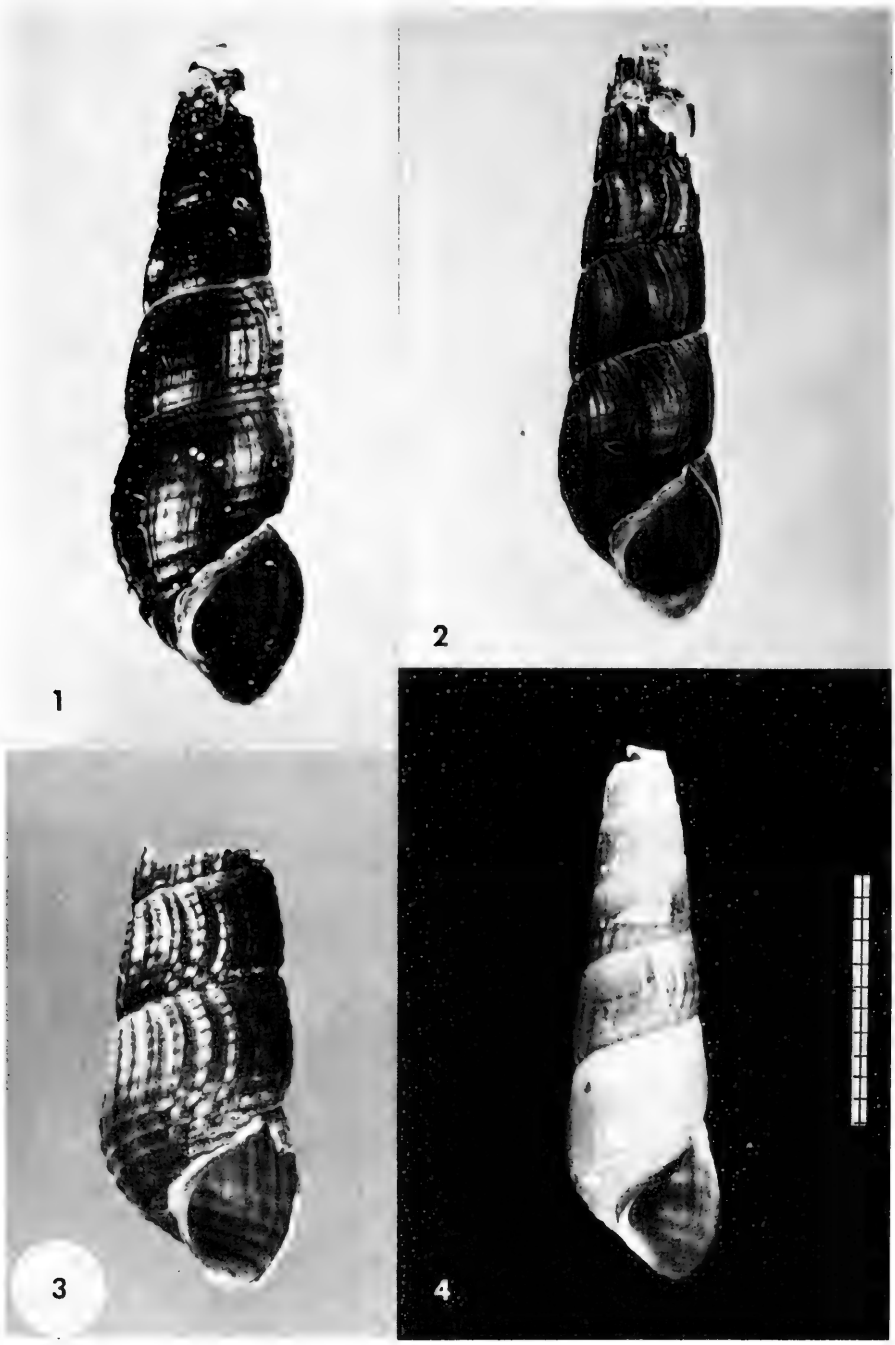


PLATE 7. Adult shells of *Semisulcospira multigranosa* (Boettger). The measurement line is in mm.

FIGS. 1, 2, 4. Three examples of the smooth morph are shown. Ribs are obsolete in Fig. 2, and only very faint elongate folds are seen in Figs. 1 and 4.

FIG. 3. An example of the ribbed morph showing the pronounced nodes on the rib. The apical whorls were removed to permit access to the gonad for cytological studies (Burch & Davis, 1967).

pendix 1. Measurements of the apical whorls are given in Appendix 2.

The shells are large (Table 38) and globose (Table 40); they are strong and ribbed. Ribs are first noticeable as low nodes at 1½ to 1¾ whorls. In early whorls (2 to 3) the ribs are often nodulate at the shoulders; in later whorls (3½) the ribs are smooth. Data on the ribs are given in Appendix 2. They are relatively wide, 0.30 - 0.48 mm in width on the second whorl. Cords do not show up until late in ontogeny, i.e., beyond 6 whorls where they cause the characteristic nodulation. At a magnification of 16×, a few faint spiral threads or grooves cross the ribs (found in some specimens of 3 to 5 whorls).

As shown in Table 32, about half of the embryos (3 whorls or larger) were uniform yellow. There were 4 banding patterns in the other embryos: (1) with subsutural, basal and sutural bands, the latter being faint and emerging at the mid-body whorl; (2) as in 1, but without the band on the mid-body whorl; (3) with only a basal band (rare); (4) only the sutural band which spirals onto the body whorl was present.

Young were removed from 27 females of shell Type 2, station 4 (24 July 1965 collection). These did not vary significantly from those discussed above in dimension per whorl or color. They had, however, more pronounced ribs.

Particularly noticeable in this species was the great variation in size of snails at the same whorl stage. For example, embryos of 4 whorls from females at station 2 measured 3.87, 4.38, 5.37 and 5.75 mm in length. As shown in Table 33, the average length was 4.71 mm for shells of 4 whorls and 5.65 mm for shells of 4½ whorls. This great variability was generally absent in other species (Appendix 1, a standard deviation for shell length, at 3.0 whorls, of about 0.1 or 0.2 mm for other species as compared to 0.3 mm for *Semisulcospira reticulata*). Many young shells looked distorted as a result of disproportionate growth.

Comparison of species

This taxon is a member of the *Semisulcospira niponica* species group. Because of the size of this species and the large number of highly noded ribs, it is not easily confused with other species. It has been compared with *S. multigranosa* in the preceeding section. The large embryos which are globose at 3 whorls are characteristic. This species has been contrasted with *S. habei* and *S. habei yamaguchi* in the sections dealing with those taxa.

Semisulcospira multigranosa
(Boettger, 1886)

Stations 3 and 6

Adults (Pl. 7, Figs. 1-4)

Statistics on basic shell features are given in Table 34. The species is polymorphic. There is shell dimorphism in that some shells are very smooth with ribs obsolete or entirely absent (Pl. 7, Figs. 1, 2, 4); or other shells prominent nodulate ribs occur (Pl. 7, Fig. 3). When the ribs are obsolete, irregularly placed low folds are observed on just 1 or 2 whorls and these fade out into elongate creases, lines or ripples, which are regularly positioned on the whorls where the ribs would normally occur. Spiral cords (7 to 8) may be pronounced, faint, or absent in the case of the smooth morphs. When absent, spiral grooves are often seen. In the multigranulate morphs the numerous ribs ($\bar{X}$, 23.2) have 7-8 distinct nodes where cords cross the ribs.

As shown in Table 35, 3 color patterns are found. These occur on both smooth and multigranulate morphs. In banded shells a central or mid-whorl stripe of yellow is flanked on either side by wide brownish-purple bands. The suture is yellow to yellowish-white.

Embryos (Pl. 11, Figs. 5-8)

Statistics on numbers of young per pallial brood pouch and the percentage

PLATE 8. Embryo shells from the pallial brood chambers of 3 species of *Semisulcospira*. The length of the scale line is 1 mm.

FIGS. 1-5. *Semisulcospira libertina*, Shimoda. Two cords are evident on the inside of the outer lip of embryos in Figs. 2 and 5. The adapical cord is the strongest.

FIGS. 6-10. *Semisulcospira libertina*. Amami-oshima. None of the embryos observed had 2 cords. One faint cord is evident in Fig. 8. About 91% of the embryos of 2.5 whorls had 1 cord. The specimen in Fig. 9 has 2.5 whorls.

FIGS. 11-15. *Semisulcospira reiniana*. Note the large size relative to embryos of *S. libertina*. The ribs terminating on a cord is characteristic.

FIGS. 16-20. *Semisulcospira kurodai*. Note the nodes on the adapical cord. The specimen in Fig. 20 has 3 whorls and 2 cords are quite evident.

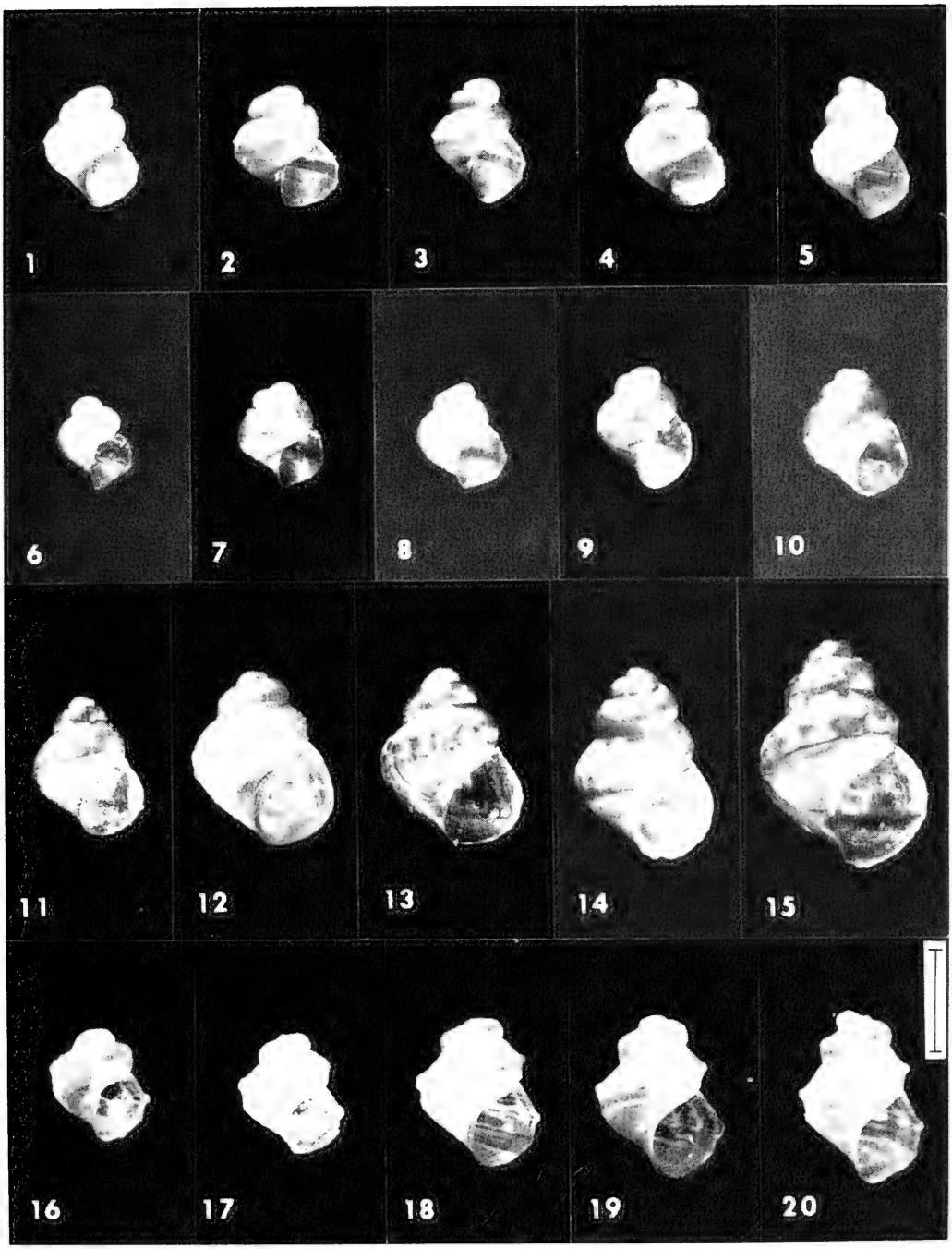

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TABLE 34. Adult shells; a statistical analysis of features of *Semisulcospira multigranosa*

No. specimens examined	Statistic	Feature measured or counted					
		Spire angle	Ribs*	Basal cords	Body whorl length (mm)	Shell length (mm)	Shell width (mm)
25	$\bar{X}$	14.2	23.2	2-3	13.1	26.0	8.3
	S	3.11	2.42	--	1.24	--	0.60
	Se	0.64	0.67	--	0.25	--	0.12

* only multigranulate forms

$\bar{X}$ = mean
S = standard deviation
Se = standard error of the mean

TABLE 35. Shell color patterns of adult *Semisulcospira multigranosa*

No. of specimens	Uniform yellowish-white (%)	Banded (%)	Uniform purple-black (%)
54	40.7	9.3	50.0

TABLE 36. Shell color patterns of embryo *Semisulcospira multigranosa*

Color of adult	No. of embryos	Uniform yellowish-white (%)	Banded (%)	Uniform purple-black (%)
Smooth morph				
yellow	16	32	25	43
banded	3	67	33	0
black	39	28	26	46
Multigranulate morph				
yellow	2	100	0	0
banded	0	0	0	0
black	0	0	0	0

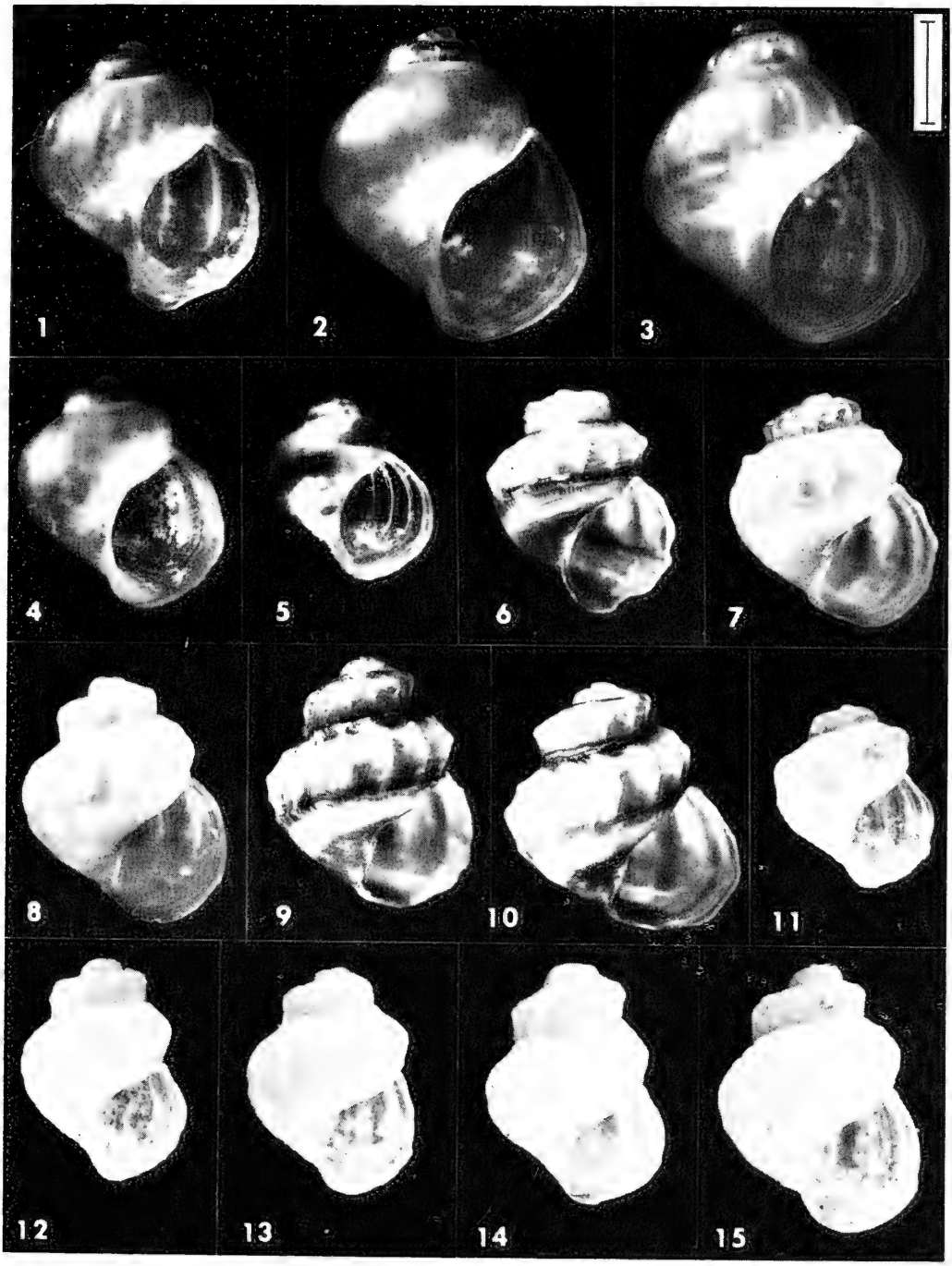


PLATE 9. The embryo shells from the pallial brood chambers of 3 taxa of *Semisulcospira*. The length of the scale line is 1 mm.

FIGS. 1-5. *Semisulcospira nakasekoae*.

FIGS. 6-10. *Semisulcospira habei*, new species. The columellar twist is particularly evident in Figs. 7, 9, 10.

FIGS. 11-15. *Semisulcospira habei yamaguchi*, new subspecies.

TABLE 37. Embryo shells; a statistical analysis of numbers per female and shell features of *Semisulcospira multigranosa*

Number females examined	Number young per female	Feature studied	Mean measurements (mm) of embryos of different whorl count							
			<2.0	2.0	2.5	3.0	3.5	4.0	4.5	5.0
16	$\bar{X}$, 5.2	Shell length			1.52	2.22	2.77	3.53	4.67	-
	S, 3.41	Shell width			1.25	1.51	1.84	2.09	2.54	-
	Se, 0.85	Body whorl L.			1.33	1.85	2.28	2.85	3.66	-
		Ratio L/W			1.22	1.47	1.51	1.69	1.84	-
	Range 2-12	% total embryos	[(<2.5)	26.0]	18.7	10.5	17.5	9.3	16.3	1.7

$\bar{X}$ = mean
S = standard deviation
Se = standard error of the mean
L = length
W = width

TABLE 38. Comparison of embryo shell length of *Semisulcospira* species at 3 whorls

Species of *Semisulcospira*	Shell length at 3 whorls			Size class
	*	**	***	
reticulata	2.59			Large
niponica	2.32(P=<.01)			
nakasekoae		2.28		
habei habei	2.23(P=<.05)	2.23		
multigranosa		2.22		
decipiens	1.83(P=<.01)			Medium
habei yamaguchi		1.78	1.78	
reiniana	1.64(P=<.01)		1.64(P=<.02)	
kurodai		1.62		
libertina	1.39(P=<.01)			Small

P= probability

* lengths in column 2 are significantly different from each other.

** the length immediately below that of the next species in column 2 or 3 is not significantly different (e.g., 2.28 of *S. nakasekoae* is not significantly different from 2.32 of *S. niponica*.)

*** lengths in column 4 are significantly different.

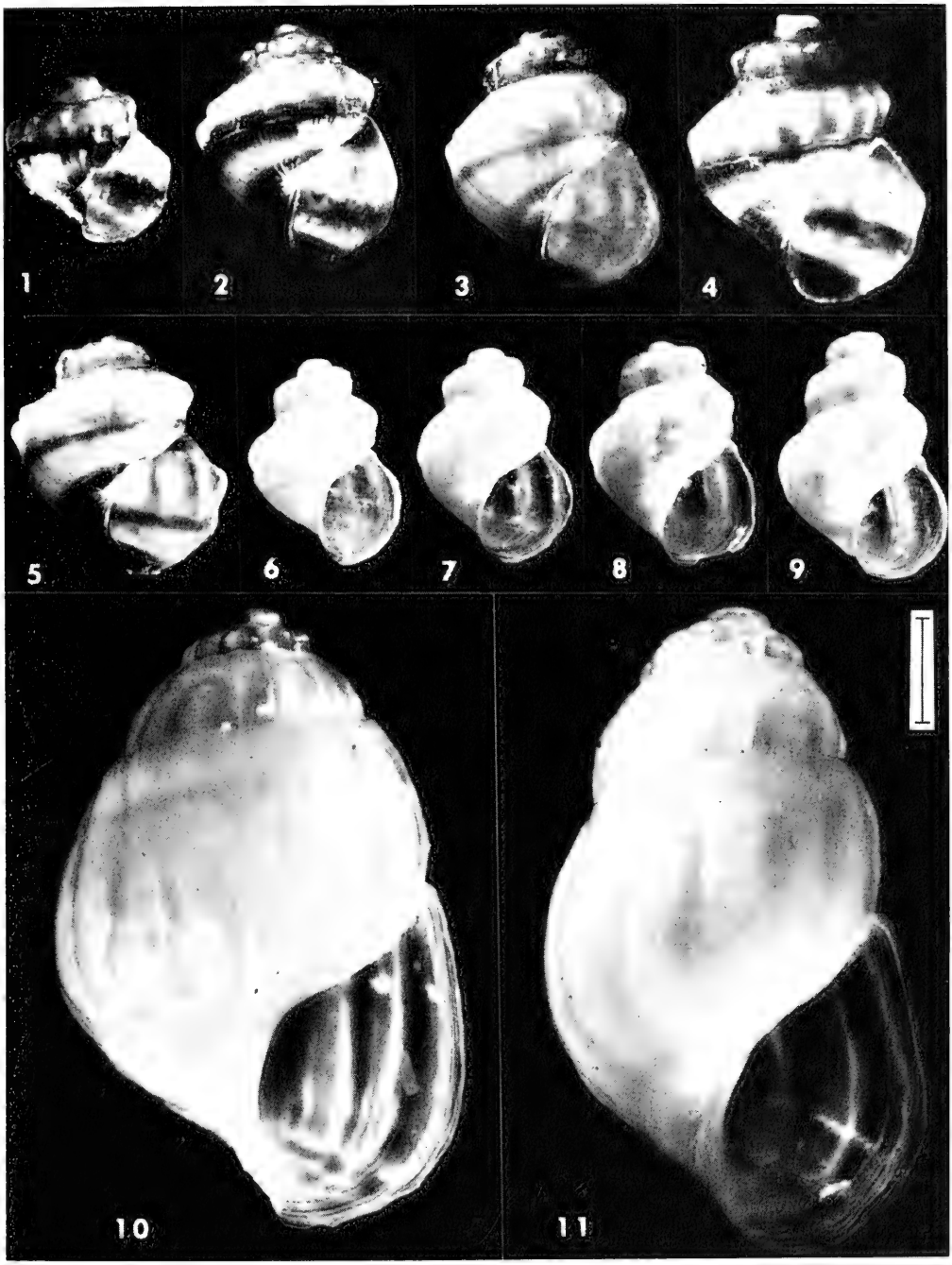


PLATE 10. Embryo shells from the pallial brood chambers of 3 species of *Semisulcospira*. The length of the scale line is 1 mm.

FIGS. 1-5. *Semisulcospira niponica*. Note the characteristic color bands in all specimens. The columella is not twisted as in *S. habei* (Pl. 9, Figs. 6-10).

FIGS. 6-9 *Semisulcospira decipiens*.

FIGS. 10,11. *Semisulcospira reticulata*.

of young at each whorl stage are given in Table 37; included are data on shell measurements. More complete statistics are given in Appendix 1. Apical whorl measurements are given in Appendix 2.

Shells are compartively large (Table 38) and elongate (Table 40). The sturdy shells have wide ribs (Appendix 2) which are not nodulate. Shells are characterized by the absence of cords and the great increase in length of body whorl after 3 whorls. The latter results in a shell with long, straight-sided whorls. Ribs first appear as low swellings on the shoulder at 1⅔ to 2 whorls. They are not nodulate and are most pronounced on the 3rd whorl. The diameter of the ribs at 4 to 4½ whorls varies from 0.29 to 0.42 mm; they are widest at mid-whorl. Shells of 4 whorls and larger have 7 ± 1 spiral grooves passing over the shells and ribs.

Embryo color patterns are given in Table 36. Too few multigranulate adults were found to determine adequately the percentage of young of each color type correlated with adult color pattern. Data for embryos from smooth as well as multigranulate adults are not sufficient to give generalities about correlation of embryo color in relation to adult color. In banded shells either a single thin basal band is present, or both sutural and basal bands occur.

Comparison of species

This species is a member of the *Semisulcospira niponica* complex. With polymorphism of this taxon in mind, it is necessary to point out criterea for contrasting this species with *S. decipiens* (done in the preceeding section), *S. habei yamaguchi* (done in the section on that taxon) and *S. reticulata*. As shown in Text Fig. 13, *S. multigranosa* has fewer ribs than *S. reticulata* (23.2 ± 2.4 as compared to a minimum of 29.0 ± 3.0). The length of the body whorl of mature *S. multigranosa* (13.1 ± 1.24 mm) is considerably shorter than that of *S. reticulata* (17.0 ± 1.3 mm), which indicates the great size differences between the taxa. There is also a vast difference in the 2 taxa when the size of the embryos at 3 whorls is compared (Table 38); *S. multigranosa* averages 2.22 mm and *S. reticulata* 2.59 mm. While some adult shells of *S. multigranosa* are black, no black *S. reticulata* shells have been found. As shown in Text Fig. 10, the embryos of *S. multigranosa* become elongate at 3 whorls, while those of *S. reticulata* are not clearly elongate until 4 whorls.

V. ANALYSES OF SHELL GROWTH PATTERNS

Species of *Semisulcospira* are characterized by shells which are elongate and conical. If the shells were true cones in the mathematical sense, the spire angle would be constant, as would be the ratio of length to width at any stage in growth. Since the shell is a coiled tube, increase in the length of the "cone" may be arithmetic or exponential; the latter because the length of the body whorl increases at a constant rate with growth.

It is clearly evident from casual observations of *Semisulcospira* that shell morphology departs from a pure cone and that increase in shell length is exponential. A series of measurements of embryonic shells from the brood pouch were taken to determine if certain departures from the ideal cone were species specific. The assumption made is that the brood pouch environment represents the most stable environment that the snail experiences, and within it occur the most uniform and regular growth processes.

With the above in mind, the following information was plotted or tabulated from data presented in the description of each species: (1) length of shell per whorl stage, (2) length of body whorl at each whorl stage, (3) the length-width ratio at each whorl stage. A number of comparisons were carried out with embryos at 3 whorls because: (1) the

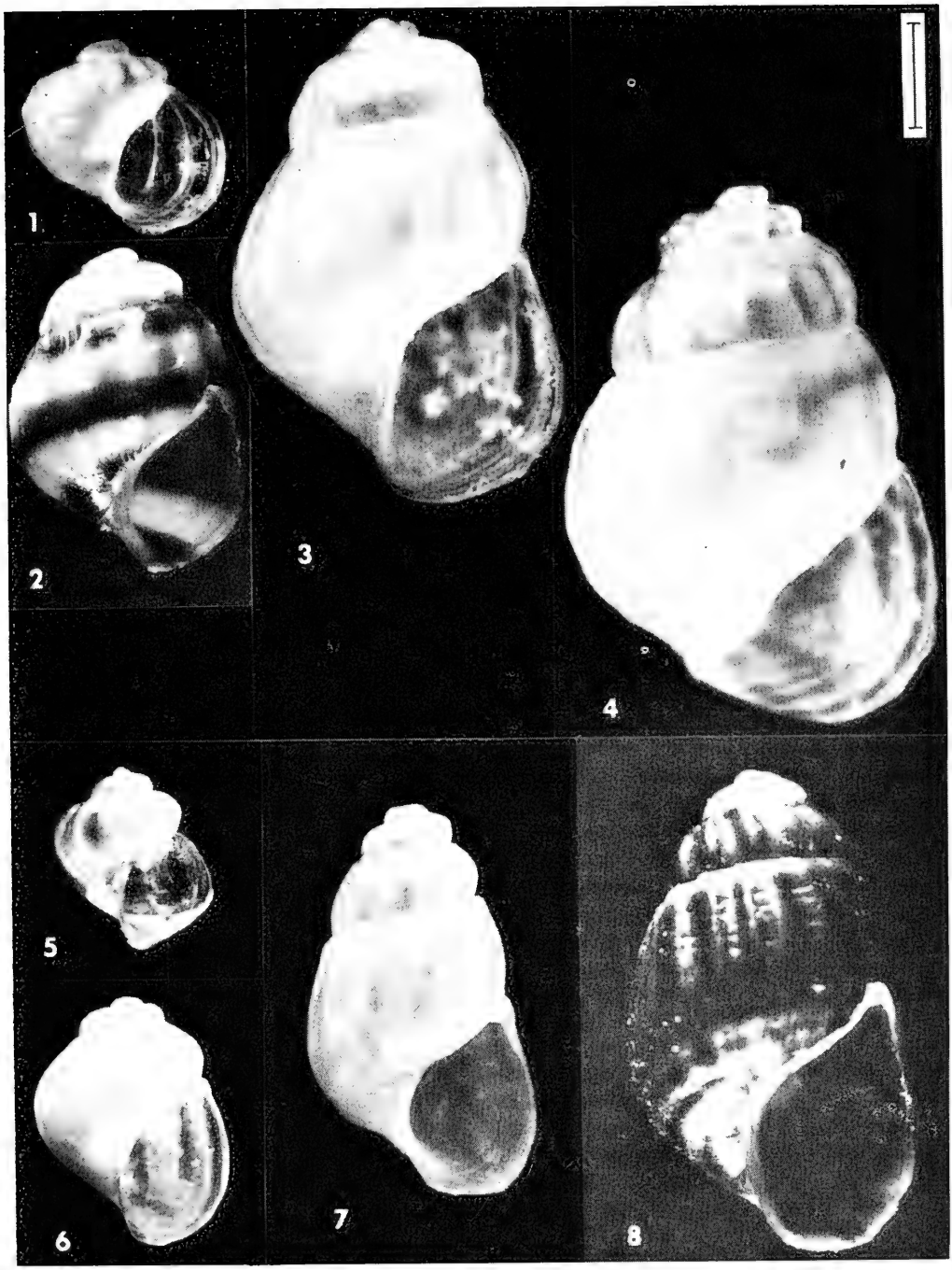


PLATE 11. Embryo shells from the pallial brood chambers of 2 species of *Semisulcospira*. The length of the scale line is 1 mm.

FIGS. 1-4. *Semisulcospira reticulata*.

FIGS. 5-8. *Semisulcospira multigranosa*.

embryos of some species reach a maximum of 3 whorls in the brood pouch, (2) this size of embryo is easily handled and measured, (3) size differences become more accentuated at this whorl stage than at lower whorl stages; (4) at 3 whorls growth is beyond the embryonic 1 or 2 whorls and the shells begin to assume progressive development to the adult shell, e.g., they become "elongate" or "globose."

Shell length per whorl. As shown in Text Figs. 5 and 6, there are, indeed, distinctive differences between some of the species in the increment of shell length per whorl. Exponential increase is generally shown where data were complete for 4 or more whorls. It is expected that those species having the lowest increments of length per whorl would demonstrate an exponential increase at later whorl stages, e.g., 4.5 and 5 whorls.

The curves for 2 groups of species do not differ significantly; these are 1) *Semisulcospira niponica*, *S. nakasekoae*, *S. habei* and *S. multigranosa* on the one hand; and 2) *S. kurodai* and *S. reiniana* on the other. The species are ranked in Table 38 by decreasing mean shell lengths at 3 whorls. They were arbitrarily grouped into size categories of large, medium and small; the divisions were indicated by gaps in the data. Data in columns 2 and 4 are for species significantly different from each other. *S. habei* is significantly different from *S. niponica* at 3 whorls and likewise the curves of length per whorl differ significantly (P=.05 level).

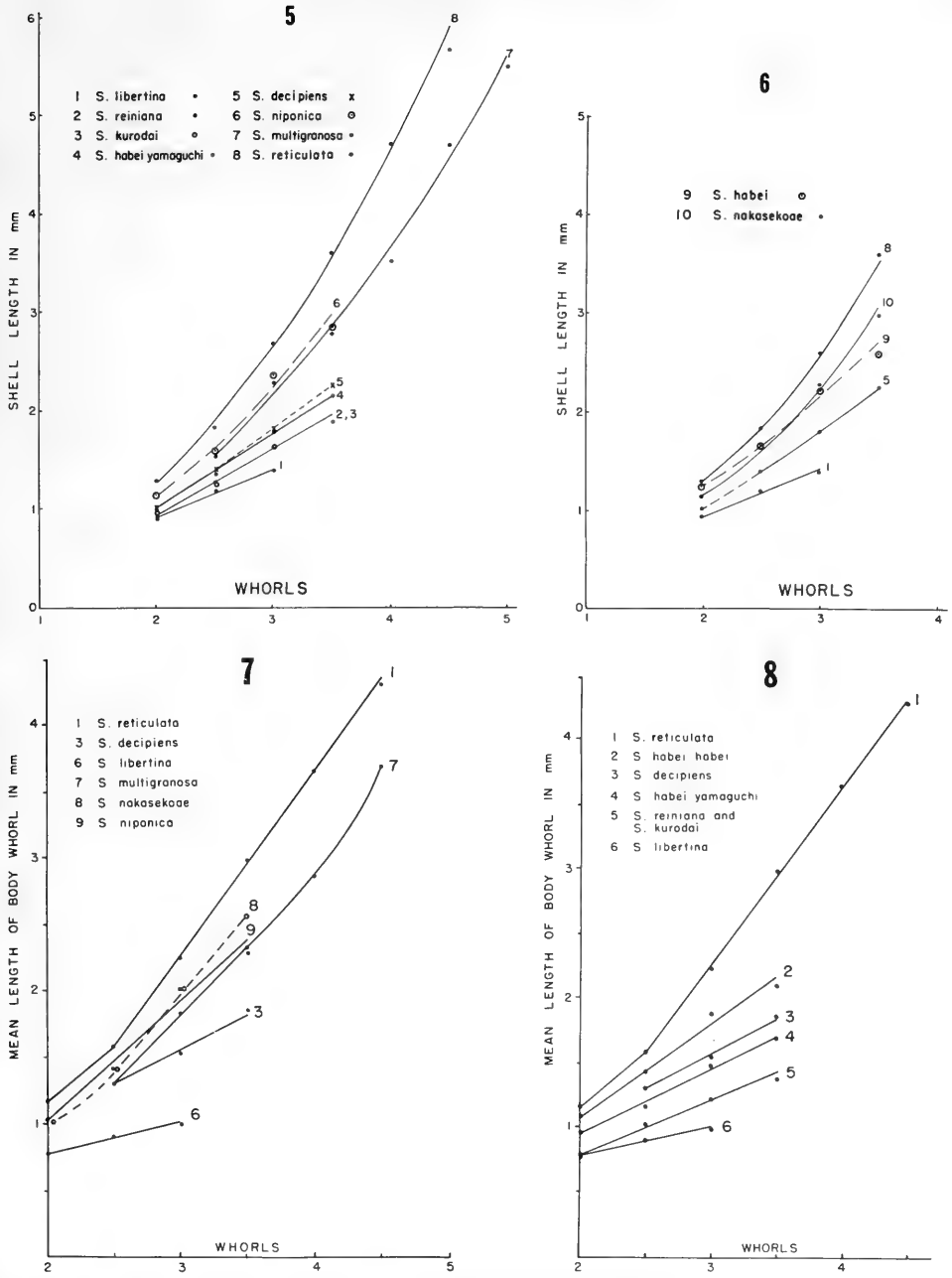
Embryos classed as "large" are 2.0 mm or longer at 3.0 whorls, those considered "medium" in length are 1.60 to 1.85 mm; those less than 1.45 mm long are "small." *Semisulcospira reticulata* is in a class by itself because of its pronounced size, while *S. libertina* is characterized by having the smallest embryos. Note the pronounced differences in size between *S. habei* and *S. habei yamaguchi*.

Length of body whorl per whorl. As shown in Text Figs. 7 and 8, there is a constant increment in the length of the body whorl at each whorl stage, as would be expected from the exponential increase in total shell length per whorl. The constant (K) increment per whorl was determined from the slope of each curve which was drawn by inspection through the data. The constants are listed for each species in order of increasing value (Table 39). Variation in plotting the curves by inspection is within ±0.10 mm/whorl. It is seen in the figures and from Table 39 that the constants for *Semisulcospira decipiens*, *S. habei yamaguchi*, *S. reiniana* and *S. kurodai* are very similar. There are at least 6 distinct slopes.

The curves fit the mean values plotted (in the figures) very well in most cases. Exceptions are the curved portions of the otherwise straight lines of *Semisulcospira nakasekoae*, *S. reticulata* and *S. multigranosa*. The slope of the lines may be altered in a significant manner due to drawing the lines by inspection only in the case of *S. nakasekoae;* it is possible that this species may have the 7th distinct slope.

Length/width ratio per whorl. The length/width ratio per whorl increases with the increasing number of whorls (Text Figs. 9 and 10). The average values are connected in the figures to clarify which points are associated with a given taxon. The reduced scale of difference between whorls accentuate the departure of points from strict linearity. Actually, the increase in ratio per whorl is fairly linear and straight lines can be drawn through the data by inspection. Constants (C) calculated from the slopes of the lines are given in Table 40.

Species are ranked in Table 40 by decreasing length/width ratios of the shells at 3 whorls. These data are used in defining the descriptive terms "elongate" and "globose." For embryos within the brood pouch, *Semisulcospira multigranosa* is an extreme form in being "elongate" with a ratio of 1.47 at 3



TEXT FIG. 5. Shell length per whorl for 8 taxa of *Semisulcospira*.

TEXT FIG. 6. Shell length per whorl for 2 species of *Semisulcospira* compared with similar data for 3 species shown in Text Fig. 5.

TEXT FIG. 7. Mean length of body whorl per whorl for embryos from 7 taxa of *Semisulcospira*.

TEXT FIG. 8. Mean length of body whorl per whorl for embryos from 3 species of *Semisulcospira* compared with 3 species shown in Text Fig. 7.

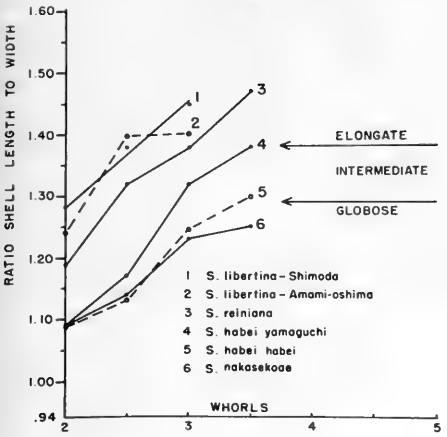
TABLE 39. The constant (k) increment of length of body whorl per whorl for species of Japanese *Semisulcospira*

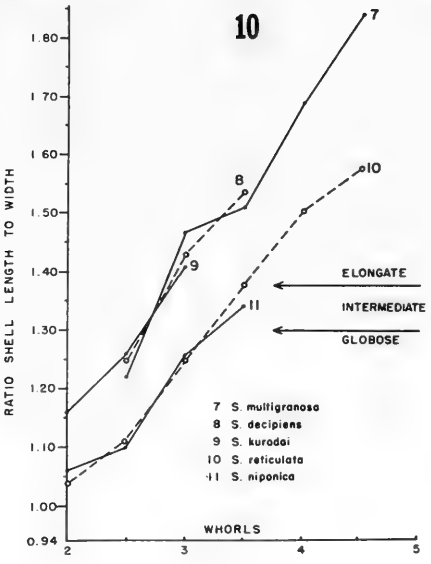
Species of *Semisulcospira*	k
libertina	0.24
reiniana	0.45
kurodai	0.45
habei yamaguchi	0.50
decipiens	0.56
habei habei	0.70
niponica	0.90
multigranosa	1.05
nakasekoae	1.20
reticulata	1.37

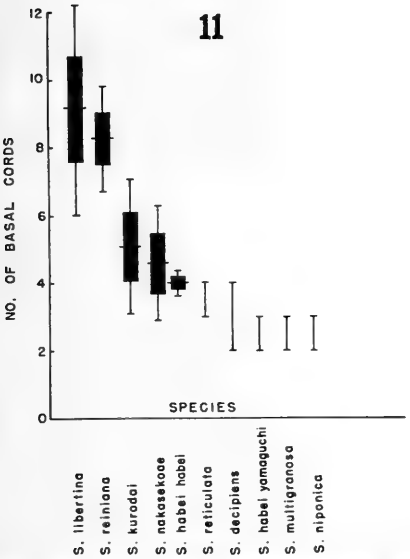
TABLE 40. Categorization of embryo shape in terms of length/width ratios at 3 whorls for species of Japanese *Semisulcospira*, and constants (C) of increments of ratio per whorl

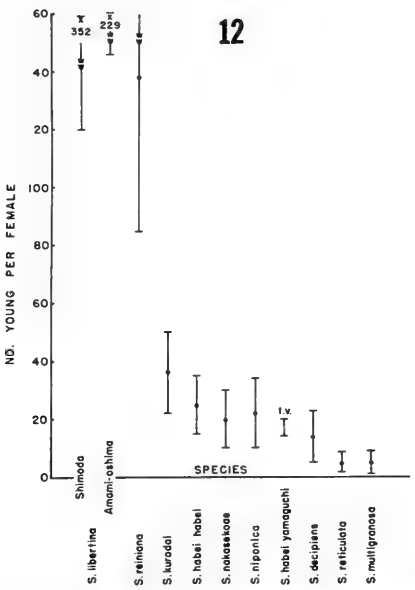
Category of shape	Species of *Semisulcospira*	Shell L/W at 3 whorls	C	Adult spire angle ($\bar{X}$)
Elongate	*multigranosa*	1.47	.33	14.2
	libertina-S.	1.45	.17	22.2
	decipiens	1.43	.33	12.4
	kurodai	1.41	.24	16.6
	libertina-A.	1.40	.21	22.6
	reiniana	1.38	.21	20.6
Intermediate	*habei yamaguchi*	1.32	.22	14.5
Globose	*niponica*	1.26	.18	20.0
	habei habei	1.25	.15	18.0
	reticulata	1.25	.23	17.0
	nakasekoae	1.23	.13	29.1

S. = Shimoda
A. = Amami-oshima
$\bar{X}$ = mean
L = length
W = width
C = constant for increment in length/width ratio per whorl









TEXT FIG. 9. Length-width ratios per whorl for embryos of 5 taxa of *Semisulcospira*. The shell shape for embryos of each whorl stage in the pallial brood chamber is indicated.

TEXT FIG. 10. Length-width ratios per whorl for embryos of 5 species of *Semisulcospira* not given in Fig. 9. The shell shape for embryos of each whorl stage in the pallial brood chamber is shown.

TEXT FIG. 11. The 10 taxa of *Semisulcospira* compared in terms of the number of basal cords on the adult shell. The mean and 2 standard deviations are shown for the 5 taxa on the left; the total range is given for the 5 taxa on the right.

TEXT FIG. 12. The 10 taxa of *Semisulcospira* compared in terms of the number of young found per pallial brood chamber. The mean and 1 standard deviation are given.

whorls. A ratio of 1.0 at the other extreme would represent a shell with length equal to width. *S. nakasekoae* comes the closest to the latter and is "globose" with a length/width ratio of 1.23. The range between 1.47 and 1.23 was arbitrarily divided into 3 categories; (1) elongate shells have a ratio of 1.38 to 1.47; (2) intermediate values range from 1.30 to 1.36; (3) globose shells have ratios between 1.20 and 1.28.

Horizontal lines in Text Figs. 9 and 10 serve to indicate the 3 shape categories and make evident at which whorl stage a species makes the transition from globose to intermediate to elongate. *Semisulcospira multigranosa* is globose at 2.5 whorls, but is elongate at 3 whorls. *S. reticulata* is globose at 3 whorls, but elongate at 3.5 whorls. At the other extreme, *S. nakasekoae* is still globose at 3.5 whorls, at which time the embryo is liberated from the brood pouch. Only 4 species make the transition from globose to elongate with a single ½ whorl increment; *S. decipiens*, *S. multigranosa*, *S. kurodai* and *S. reticulata*. These are species with the highest increment constants.

Constant increment in ratio indicates that the spire increment angle is continually decreasing through ontogeny. Those species with pronounced large constants (C) are characterized by slender adult shells with small spire angles (e.g., *Semisulcospira multigranosa*, *S. decipiens*, Table 40 Text Fig. 10). As seen in Table 40 there is only a general tendency for an increase in constant to be correlated with a decrease in adult spire angle; the coefficient of correlation, r, is -0.66 and is significant ($P=<.05$).

VI. DISCUSSION ON THE UTILITY OF CHARACTERS, WITH A KEY TO THE SPECIES

Grouping characters in terms of importance

The characters or traits used in this paper to describe 9 species and 1 subspecies on the Japanese genus *Semisulcospira* are listed in Table 41 in order of decreasing value for characterizing the specific level or species groups. Assurance that these taxa were genetically distinct was based on the cytological data of Patterson (1967a,b), Burch & Davis (1967), and Burch (1968), coupled with major adult and embryo shell features. Cytological complexity within the genus *Semisulcospira* of Japan (Table 42) provides adequate criterea for the objective delineation of the species discussed here. With this objective framework it is possible and worthwhile to rank characters according to their usefulness in describing species in terms of basic features readily observed.

The characters are placed in 4 groups, referred to here as primary, secondary, tertiary and quaternary. Three of these represent characters governed by segments of the genotype which are operative at different levels; the supraspecific, specific and infraspecific. The last group of traits is that where the environment masks inherent genetic potential.

Primary characters are those which serve to define groups of species. They also serve to define, in part, the specific level. Theoretically, the species placed in a group are phylogenetically more closely related to each other than to species of another group. Primary traits pertain to all the taxa of a genus and are of first ranked importance in justifying specific status for a taxon. They are obviously important key characters because they serve to separate taxa in terms of non-overlapping variation (i.e., mean and standard deviation).

Secondary characters are of use in defining the species and also provide good key characters. Secondary characters, while naturally variable, should not be subject to such inter-population variation that there is confusion in species recognition. For example,

TABLE 41. Characters ranked in order of decreasing value for describing the specific level of species groups

Characters	Major groups of characters
1. Chromosome number 2. Adult; number of basal cords 3. Adult; number of embryos per female	Primary (species groups)
4. Ontogeny of ribs 5. Adult; number of ribs and nodes 6. Embryo, sizes and shapes 7. Embryo; increment of dimension or ratio per whorl 8. Embryo; greatest whorl stage in the pallial brood pouch 9. Embryo; number of ribs and nodes 10. Embryo color patterns 11. Embryo; cords 12. Adult; shell width 13. Adult; shell spire angle	Secondary (species level)
14. Adult; length of body whorl 15. Embryo; microsculpture 16. Embryo; apical whorl measurements 17. Adult; color patterns	Tertiary (subspecific level) = population variation
18. Adult; shell length 19. Adult; number of whorls	Quaternary (environmental)

*Traits 11 to 14 overlap in the secondary-tertiary grouping

TABLE 42. Data from Burch (1968) on the chromosome numbers of species of Japanese *Semisulcospira*

Species of *Semisulcospira*	Inferred basic haploid number	Observed variations of the diploid number
libertina	18	36
reiniana	20	40
kurodai	18	35,36
nakasekoae	13	26
**habei habei*	7	17–20
**habei yamaguchi*	7	17–20
niponica	12	25–27
decipiens	12	25,26
reticulata	12	25,26
multigranosa	14	28–31

*These were not discussed by name in Burch, 1968

the "presence or absence of ribs" would make a poor secondary character. This is shown in *Semisulcospira libertina* where populations from Amami-oshima had no evidence of ribs while the same species from Shimoda had shells with ribs in 3% of the population. In more recent studies (Davis 1967b, 1969), I investigated a population from Ashino Lake in the Hakone mountain range, Kanagawa Prefecture where all the snails had distinct ribs. This population is considered conspecific with *S. libertina* because of its agreement with topotypes of *S. libertina* in primary and secondary characters (also electrophoretically and immunologically, Davis, *loc. cit.*). Martens (1877) noted the gradation from shells with no ribs to those with pronounced ribs when he studied populations of *S. libertina* and he accordingly gave variety names to the stages, such as *tenuisulcata*, *ambidextra*, *decussata* and *plicosa*.

A species is defined in terms of unique mixtures of secondary traits, grouped within the framework of primary characters. Traits ranked closer to the primary characters in Table 41 indicate where more distinct breaks in the data occur when all the taxa are compared using a single trait. For example, when the taxa are compared in terms of numbers of ribs on the penultimate whorl (Text Fig. 13) more distinct groups of data are seen than when data for spire angles are compared (Text Fig. 18).

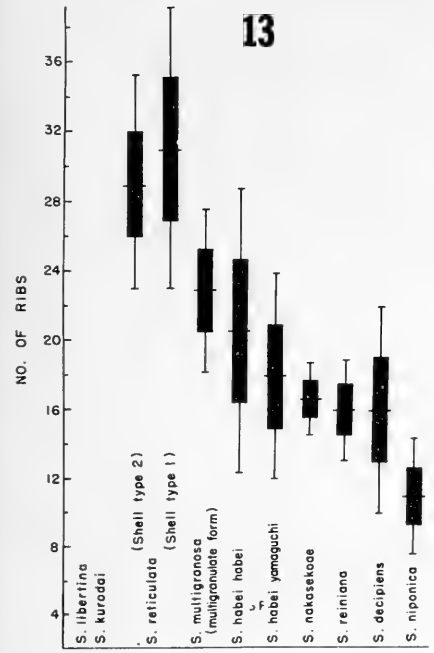
Tertiary characters are those which vary a great deal from population to population. As shown by the bracketline in Table 41, traits 11 to 14 tend to be affected by population variation. While, for example, it is clear that *Semisulcospira nakasekoae* and *S. decipiens* are distinct when they are compared on the basis of spire angle (Text Fig. 18), the degree to which the environment affects growth patterns of snails of different populations of the same species is not clear, and, in particular, how shell width and spire angle are affected. In the case of cords on the embryo, *S. multigranosa* has none, while *S. libertina* is characterized by their presence. Yet, *S. libertina* from Shimoda had 2 cords (in 70% of the individuals with 3 whorls), while embryos of the same species from Amami-oshima had only 1 (in 91% with 2.5 whorls). The number of cords varies between populations.

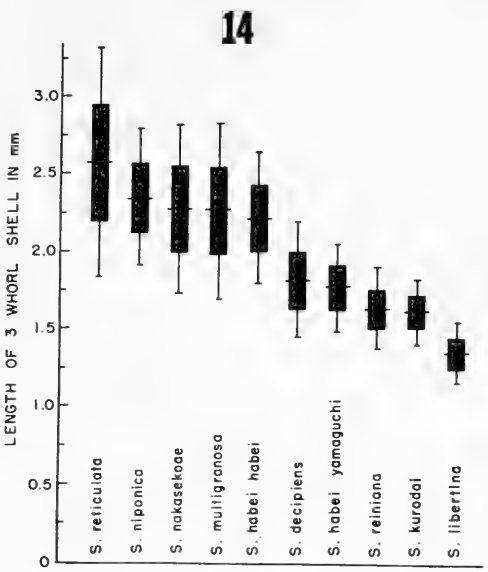
A character in this group may have similar types of expression in many taxa yet serve to characterize 1 species. The color patterns of adult shells for several taxa vary from uniform yellow to banded in similar patterns; however, *Semisulcospira niponica* is characterized by having a dark brown-black to purple black shell.

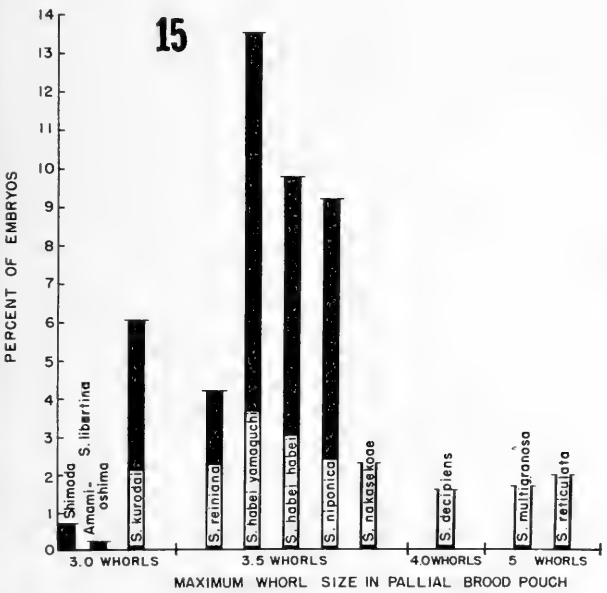
Quaternary characters are those which are acted on by the environment to the extent that the genotype is not expressed. Characters involving adult shell size are generally poor because of lack of information on how different environments affect growth.

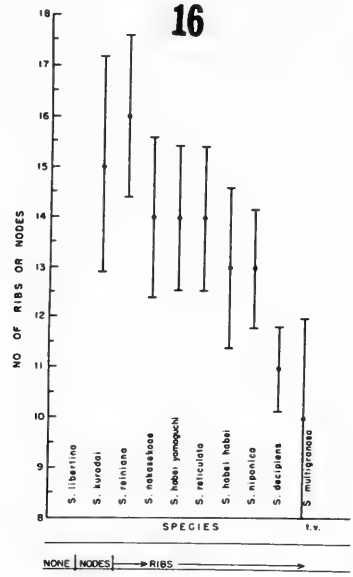
Key to the species of *Semisulcospira*

The following key is presented to show how groups of primary and secondary characters serve to distinguish the species of this study. Only the most easily observed characters are employed. By necessity the key must be used in connection with data provided in the tables for each species, and Tables 38 and 40 and Text Figs. 5 to 19. At least 10 to 25 adult specimens are needed and these must be in good condition; embryos from 5 or more females are needed; and, averages of data should be used. Traits of chromosome numbers and indexes of increment of length per whorl for the embryos are not used because they are comparatively difficult and/or tedious to work out, thereby offsetting the purpose of the key which is the rapid and efficient identification of species. The key serves to show which groups of readily observed characters are indicative of distinctive









TEXT FIG. 13. The 10 taxa of *Semisulcospira* compared in terms of the number of ribs on the penultimate whorl of adult shells. The mean and 2 standard deviations are given. Shell type 2 of *S. reticulata* conforms to the type; shell type 1 has the convex body whorl. [*S. libertina* does not have ribs on shells from the populations studied; *S. kurdoai* does not have ribs on the penultimate whorl of mature adults.]

TEXT FIG. 14. A comparison of the 10 taxa of *Semisulcospira* in terms of embryo shell length at 3 whorls. The mean and 2 standard deviations are given.

TEXT FIG. 15. The maximum embryo whorl size found in the brood chamber of each taxon of *Semisulcospira* and the % of embryos attaining that size.

TEXT FIG. 16. The number of ribs or nodes on the most apical whorl of the embryo having these structures is given for each taxon of *Semisulcospira*. The mean and 1 standard deviation are given for 8 taxa while the total variation (t.v.) is given for 1 taxon.

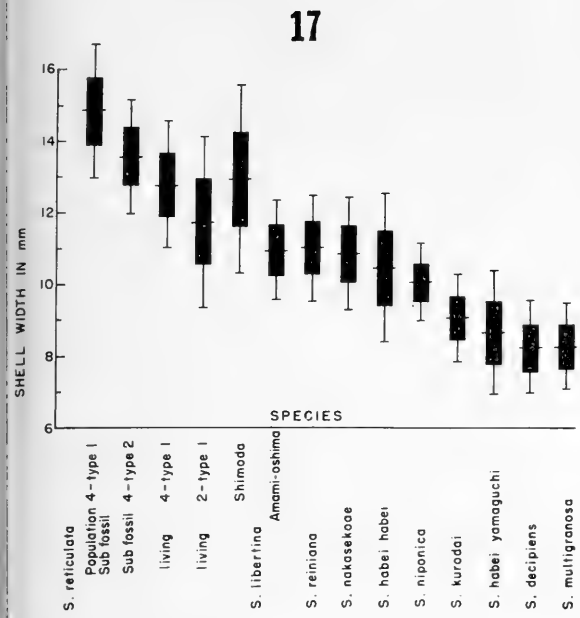
genotypes.

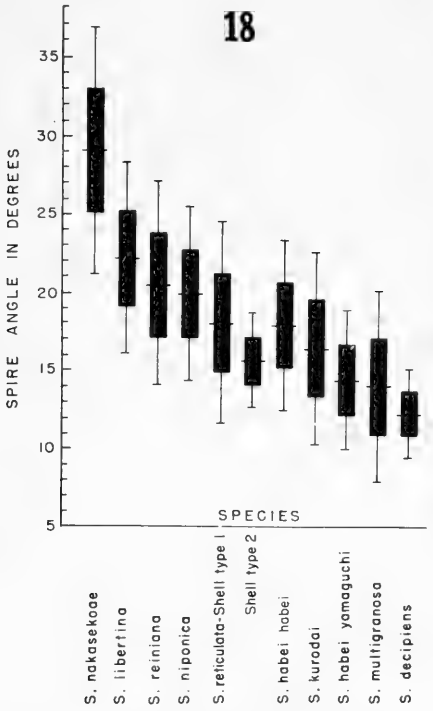
The key is limited in use until more populations of *Semisulcospira libertina* are investigated, because (1) this species seems to be the most widespread and variable of the species studied, and (2) numerous named species or varieties were placed in symonymy under *S. libertina* by Kuroda (1963) and the possibility does exist that some of these, e.g., *S. nassaeformis* and *S. kawamurai*, may be good species.

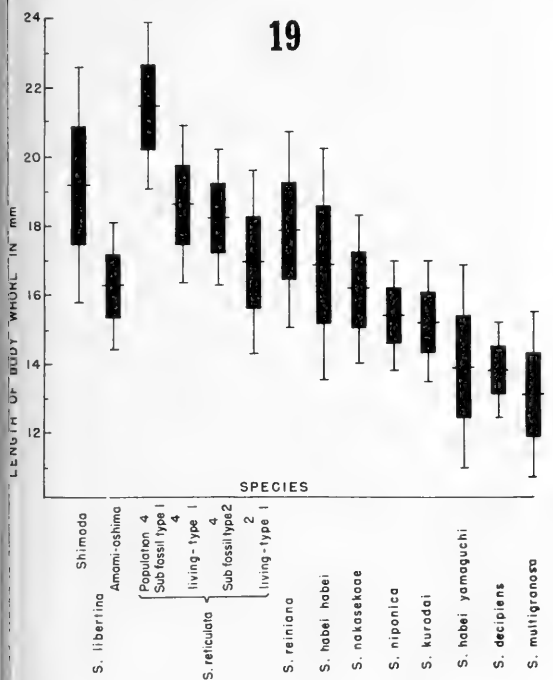
1a. Basal cords average 8 to 10; more than 80 embryos per pallial brood pouch. 2
1b. Basal cords average 6 or less; fewer than 60 embryos per pallial brood pouch. 3
2a. Embryos small, without ribs . . *S. libertina*
2b. Embryos medium in length, with pronounced ribs . *S. reiniana*
3a. Adults without ribs on the penultimate and body whorls; ribs, when present are on 1 or 2 apical whorls only 4
3b. Adults with distinct ribs, from early whorls to the body whorl. 6
4a. Adult spire angle from 12 to 16 degrees; basal cords 2 or 3; embryos large, elongate and with up to 4½ to 5 whorls in the brood pouch . . . . . *S. multigranosa* (smooth morph)
4b. Adult spire angle from 15 to 34 degrees; basal cords 3 to 6; embryos large or medium in length, elongate or globose, and with up to 3½ whorls in the brood pouch 5
5a. Adult spire angle 24 to 34 degrees; embryos large and globose . . . . . *S. nakasekoae*
5b. Adult spire angle 14 to 21 degrees; embryos medium in length and elongate. . . .*S. kurodai*
6a. Ribs on adults 9 to 13 with 3 or 4 large pustulate nodes on each . . . . . *S. niponica*
6b. Ribs on adults 13 to 37 with 5 or more nodes per rib 7
7a. Adults with 20 to 37 ribs on the penultimate whorl (averaging 22 to 32); embryos up to 4½ or 5 whorls in the brood pouch. . . 8
7b. Adults with 12 to 24 ribs on the penultimate whorl (averaging 14 to 22); embryos up to 3½ whorls in the brood pouch 9
8a. Embryos globose, with 13 to 15 ribs; adults with 26 or more ribs (averaging 28 or more) on the penultimate whorl, with 5 to 7 nodes per rib . . . . . *S. reticulata*
8b. Embryos elongate, with 8 to 12 ribs; adults with 26 or less ribs (averaging 25 or less) on the penultimate whorl, with 7 to 8 nodes per rib . . *S. multigranosa*
9a. Embryos large and globose; adults without external banding . . . . . *S. habei habei*
9b. Embryos of medium length and elongate or intermediate in shape; adults with or without external color bands. 10
10a. Ribs of adults smooth to weakly nodulate, 7 to 8 nodes per rib; embryos with 10 to 12 ribs beginning at 1¾ to 2 whorls; embryos elongate. . . .*S. decipiens*
10b. Ribs of adults distinctly nodulate, 5 to 6 nodes per rib; 13 to 15 ribs on the embryo, beginning at 1¼ to 1¾ whorls; embryos intermediate in shape. . . *S. habei yamaguchi*

Discussion on the utility of characters

It is noted in the above key that basal cords and numbers of embryos per brood pouch separate *Semisulcospira libertina* and *S. reiniana* from the remaining species. As seen in Text Figs. 11 and 12, further gaps in the data for these traits are seen in other species. *S. kurodai* is clearly in an intermediate category when both basal cord number and numbers of young per female are concerned. The above 3 taxa are united into a group by having similarly high







TEXT FIG. 17. The 10 taxa of *Semisulcospira* compared in terms of adult shell width. Two populations of *S. reticulata* are compared involving 2 different shell types. Shell type 2 conforms to the "type," while shell type 1 has more convex whorls. Two populations of *S. libertina* are compared. The mean and 2 standard deviations are given.

TEXT FIG. 18. The 10 taxa of *Semisulcospira* compared in terms of adult shell spire angle. Shell type 2 of *S. reticulata* conforms to the "type," while type 1 has pronounced convex whorls. The mean and 2 standard deviations are given.

TEXT FIG. 19. The 10 taxa of *Semisulcospira* compared in terms of length of body whorl of the adult shell. Two populations of *S. libertina* and *S. reticulata* are compared. The latter involved type 2 shells conforming to the "type," and type 1 shells with pronounced convex whorls. The mean and 2 standard deviations are given.

chromosome numbers of n=18 or 20 (Table 42). This group is called the *"S. libertina* species complex." The other species group, the *"S. niponica* complex," is characterized by low chromosome numbers (n=7 to 14), low numbers of basal cords (mean numbers of 2.5 to 5), and comparatively few embryos per female (mean numbers of 5 to 25).

At this point, secondary characteristics become important in discriminating between species. As discussed above, the presence or absence of ribs is not a good secondary character; however, the ontogeny of ribs is excellent. Both *Semisulcospira kurodai* and *S. nakasekoae* have a particular development and morphology of rib not seen in other species. The early cessation of rib formation in the former is particularly striking. With the exception of *S. nakasekoae* and the smooth morph of *S. multigranosa*, other members of the *S. niponica* complex have well developed ribs throughout ontogeny.

As seen in Text Fig. 13, the number of ribs on the penultimate whorl serves to distinguish several species of the *Semisulcospira niponica* group. It is evident at a glance that *S. reticulata* has significantly more ribs than *S. multigranosa*, and that *S. niponica* has significantly fewer ribs than *S. decipiens*. Similarly, *S. multigranosa* has significantly more ribs than *S. habei yamaguchi*, *S. nakasekoae* and *S. decipiens*. The number of nodes on the ribs of these species characterizes some of the species. *S. niponica* has 3 or 4; *S. decipiens* and *S. multigranosa* have 7 or 8; *S. habei* and *S. habei yamaguchi* have 5 or 6.

Embryo size and shape vary considerably between species, as shown in Tables 38 and 40, and Text Fig. 14. These differences are accentuated when plots are made of increments of dimension per whorl (Text Figs. 5 to 8). The curves in these figures demonstrate significantly different patterns of development in the female brood pouch. Consider the very significant difference between the curves for length of shell per whorl when data from *Semisulcospira libertina*, *S. decipiens* and *S. multigranosa* are plotted (Text Fig. 5). It is also evident in these figures that 2 or more species may have similar developmental patterns. For example, *S. reiniana* and *S. kurodai* are identical in terms of mean length per whorl. *S. niponica* and *S. multigranosa* have similar curves for length of shell per whorl, but the former closely resembles *S. reticulata* in terms of increment of length/width ratio per whorl (Text Fig. 10). The feature of greatest whorl stage attained by an embryo in the brood pouch (Text Figs. 5-10, 15) often serves to separate those taxa with otherwise similar curves and to characterize each species.

Embryos reach a maximum whorl stage in the brood pouch of 3 to 5 whorls, depending on the species. The percentages of embryos reaching the maximum whorl stage is given for each species in Text Fig. 15. Generally, larger numbers of whorls are associated with fewer and larger sized embryos in the brood pouch. It is not known whether or not embryos of a given taxon at a maximum whorl stage (Text Fig. 15) may at times gain another half whorl before birth, expecially when a large percentage (8% or more) have reached the maximum size. I have collected *Semisulcospira libertina* from Shimoda throughout the year and have always found the female brood pouch full of embryos no larger than 3 whorls. However, as it may be possible for a species such as *S. habei yamaguchi* to have embryos with 4 whorls, I have used this character to separate taxa on the basis of at least 1 whole whorl difference.

Gross sculpture on the embryos is an important secondary character. There are taxa with no nodes or ribs, taxa with nodes, and taxa with ribs (Text Fig. 16). Taxa without ribs are prominent in the *Semisulcospira libertina* complex, but *S. reiniana* is the

exception with well developed embryonic ribs. *S. libertina* from both localities has smooth embryos. In later studies (Davis, 1967b, 1969), I found 1 embryo from a female from Shimoda which had distinct nodes on the shoulder of the second whorl and the nodes were elongated into ribs. In the afore mentioned population at Ashino Lake, the embryos of the prominently ribbed adults had well developed nodes at the shoulder of the whorl. In some of these, ribs developed by the elongation of the node. *S. kurodai* is characterized by embryos with nodes which occasionally elongate into ribs. The embryos of these taxa are also similar in that they possessed 1 or 2 spiral cords. Not all embryos generally characterized by nodes or ribs have spiral cords. In *S. reiniana*, for example, 20% of the embryos at 3 whorls lacked ribs.

In the *Semisulcospira niponica* complex the number of ribs on the initial ribbed whorl of the embryo shell helps in distinguishing taxa. *S. multigranosa* and *S. decipiens* have significantly fewer ribs per volution than *S. niponica* and *S. habei* (Text Fig. 16). Using data in Appendix 2 it was found that *S. habei* has significantly fewer ribs than *S. reticulata*, *S. habei yamaguchi* (P=.01) and *S. nakasekoae* (P=.05). The fact that this character serves to distinguish between *S. habei yamaguchi* and *S. decipiens* is most helpful, since the adult shells look very much alike (similar widths, Text Fig. 17; similar spire angles, Text Fig. 18; similar lengths of body whorls, Text Fig. 19; etc.).

Embryo color patterns are useful in characterizing several species. Brownish embryos characterize *Semisulcospira libertina* and *S. reiniana*. *S. kurodai* has glassy or opaque white shells which may have a yellow or brown basal band on shells of 3 whorls. *S. niponica* has a globose shell with 3 very distinct purple-brown bands. Some embryos of *S. multigranosa* are a uniform purple-black.

Embryos of most taxa have variable color patterns, and therefore the use of color patterns to discriminate between such taxa is best made when other traits are considered concomitantly. For example, *Semisulcospira reticulata* is characterized by having large and globose embryos with shell color patterns of either uniform yellow or banded; none are purple-black. Embryos of 5 whorls are found in the pallial brood pouch.

The presence or absence of cords in populations of *Semisulcospira libertina* was discussed above. *S. kurodai* and *S. libertina* (Shimoda population) both have 2 distinct cords. *S. reiniana* has ribs which terminate on a pronounced cord. Spiral cords on the embryos tend to characterize the *S. libertina* complex. Cords are present or lacking on embryos of species in the *S. niponica* complex. They are not found on embryos of *S. nakasekoae*, *S. multigranosa* and *S. reticulata* (under 6 whorls). *S. habei habei* may or may not have a cord, which, when present, may be either smooth or nodulate. A low cord is found on *S. niponica*. A noded cord is frequently found on embryos of *S. decipiens*.

Traits used to delineate the specific level become less useful when they vary qualitatively from population to population, or when quantitative characters are based on significant differences where P is at the .05 level and/or measurements vary by very small amounts (e.g., apical whorl measurements). With the exception of *Semisulcospira nakasekoae*, statistical analyses must be used to test for significant differences between taxa ranked next to each other by decreasing spire angle, width, or length of body whorl (Text Figs. 17-19). The characters of spire angle or shell width (Text Figs. 18 and 17) are useful as key characters where extremes are separated, e.g., in separating *S. multigranosa* or *S. decipiens* from *S. habei* or *S. niponica*.

It is important to note that shell types 1 and 2 (shells of living shell

type 1, and shells of subfossil type 2, station 4) of *Semisulcospira reticulata* had significantly different spire angles (P=.02) and widths (P=.02), although there were no significant differences in lengths of body whorl (i.e., size), numbers of ribs, or nodes on the ribs. Differences in length of body whorl between populations of *S. libertina* and populations of *S. reticulata* indicate differences in degree of growth (population differences). The same holds true for differences in width between populations of *S. libertina* and populations of shell type 1 of *S. reticulata* (Text Fig. 17).

Adult color patterns are extremely variable between populations of the same species. For example, *Semisulcospira libertina* from Amami-oshima all had uniform yellow shells, while those from Shimoda had either uniform yellow or banded shells. *S. libertina* from Ashino-lake had uniform purple-blue, uniform yellow, and banded shells. The banding patterns are variations of a basic pattern of basal, mid-whorl and subsutural bands, where 1 or more bands are often missing or vague. The bands may be a brighter red-purple in some and brown-purple in other populations. Similar banding patterns are seen in other species, e.g., *S. reiniana*, *S. kurodai*, *S. habei yamaguchi*.

A few species have distinctive color patterns. For example, there are no light colored shells in *Semisulcospira niponica*, and the general purple-black or brown-black shell color tends to mask the banding present on some shells. *S. reticulata* has shells that are either uniform yellow or banded. The banded shells are distinct in having a purplish-brown band at mid-whorl.

Semisulcospira multigranosa and *S. decipiens* not only look quite similar, but also both have shells of 3 color patterns: uniform yellow, banded and uniform purple-black. In both species, banded shells have a yellow stripe at mid-whorl flanked on either side by purplish-brown bands.

Microsculpture appears to vary, partly with the population. For example, *Semisulcospira libertina* from Shimoda has a distinct cancellate microsculpture not seen on specimens from Amami-oshmia.

Apical whorl measurements are listed in Appendix 2. Unless the measurements are made exactly as demonstrated by Davis (1967a), the mean values may vary significantly from those given. As the order of magnitude of difference between taxa is not very great, and as the measurements are tedious to make, as well as subject to great variation if not done as described, I consider this character of somewhat limited value. The populations of *Semisulcospira libertina* did differ significantly in the width of the apical whorl. The difference between the diameters of the apical whorls of *S. nakasekoae* and *S. reticulata* was 0.14 mm, and the standard error of the mean of each did not exceed 0.009 mm; it is therefore evident that a distinct order of magnitude of difference exists between these two species. Significant differences exist between the mean values for a number of taxa, but I do not consider these differences especially relevant as diagnostic characters, because the order of magnitude of difference between the means is so small.

Characters environmentally controlled.

The length of the shell and the number of whorls have little meaning when the degree of erosion of the apical whorls is clearly associated with environment. Populations of *Semisulcospira libertina* from numerous areas have been observed and shells from these varied habitats have ranged from nearly entire with 7 to 8 whorls, to severely eroded shells with only the last 3 whorls remaining. It would seem desirable to investigate the relationships between environmental water pH, chemical composition of the water, and growth phenomena in *S. libertina* (rate of growth, degree of shell erosion, width of shell, spire increment angle and length of the

body whorl).

VII. CONCLUDING DISCUSSION

Taxa not studied.

This paper probably covers most of the species of *Semisulcospira* found on the 4 main islands of Japan, even though 17 nominal taxa listed in Table 1 are not discussed here. Nevertheless, several of the latter may be distinct species. In this regard, *S. libertina nassaeformis* Kuroda & Kanamura (in Kuroda, 1929), *S. kawamurai* Kuroda (1929) and *Melania reiniana* var. *hidachiensis* Pilsbry (1902) should be studied because of their interesting adult shell characters. In fact, each named taxon should be properly accounted for by studying the topotype population to ascertain if it is a valid species or merely a synonym of another species, and if synonymy is justified, then adequate reason should be given. A number of the nominal species which were not studied were those for which no type locality is known. Others were varieties considered by their authors to be within the species concept of *S. libertina* (e.g. varieties *decussata, plicosa*, and *irrigua* of von Martens, 1877). In the future, I plan to study as many of these nominal taxa as possible.

Identification of species

Concepts for the species presented here were taken from their original descriptions, and expanded on from a study of topotype populations (except for *Semisulcospira reiniana*). Dr. T. Habe, Natural Science Museum, Ueno Park, Tokyo, pointed out to me the topotype populations for *S. kurodai*, *S. nakasekoae* and *S. reticulata*.

The polymorphic nature of *Semisulcospira multigranosa* is noted here for the first time. Specimens of the ribbed morph were sent to Dr. Zilch, Senkenberg Museum, Frankfurt am Main, Germany, who compared them with the type material and thus verified the identification. *S. multigranosa* corresponds, in part, to the ribbed morph figured and discussed by Kajiyama & Habe (1961). The species figured by Kawamura (1918) as this species actually conforms to the type of *S. reticulata*.

Semisulcospira libertina (Gould) was readily located and identified at the Shimoda locality. *S. niponica* (Smith) and *S. biwae*[1] (Kobelt) were readily obtained and identified from Lake Biwa (see figures in Brot, 1874, Pl. 34, Fig. 10; Kobelt, 1879, Pl. 19, Fig 9). *S. decipiens* discussed here corresponds to the specimen figured by Kajiyama & Habe (1961).

Semisulcospira reiniana was described by Brot but the description was first published by Kobelt (1876). No type locality was given. Brot (1874)[2] gives the type locality as "Yokohama (v. Martens)." Martens (1877) described *S. libertina* var. *irrigua* from Yokohama, Japan and this taxon is discussed by Kobelt (1879) under "*Melania Reiniana*." Boettger (1886) studied what he considered to be *S. reiniana* from a brook near Lake Biwa, and which he considered to agree with Brot's illustration of *S. reiniana* (Brot, 1874, Pl. 34, Fig. 14, 14a); Boettger stated that to his knowledge *S. reiniana* previously was known only from "Yokohama (Rein)." The specimens of the population I studied agreed with the original description and the figures given by Kobelt (1876, Pl. 8, Fig. 4; 1879, Pl. 18, Fig. 2).

Reasons for studying the largest 10% of each population.

I have described the species I studied

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[1]This variety is synonymous with *Semisulcospira niponica*, it is simply an extreme form in which the nodules are remarkedly pronounced.

[2]Brot evidently wrote his "Die Melaniaceen" in sections, the last parts apparently were written after the Kobelt paper in 1876 describing *S. reiniana*.

in terms of adult shells and embryos. I used the largest 10% of the population collected to represent the adult condition because (1) material currently present in museums is comprized of the largest examples of a population (for the most part), (2) classical descriptions involve the largest specimens, and (3) criteria for establishing the adult or mature condition in *Semisulcospira* have not been adequately established. The main reason is the latter one. No data have been published which give information on the population structure of any species of *Semisulcospira* so that the following are known: (1) the growth rate of newly born individuals, (2) mortality schedules for the young born, (3) the size and age at which the snails become sexually mature, (4) the sex ratio of the population, and (5) the production of young per female per unit time.

Over a 3 year period, I have observed that many populations of *Semisulcospira* in Japan appear to be in a steady state with regard to the size composition of the population and the maximum size of individuals. When one rapidly collects by hand-picking 1000 specimens from a large population, it is inevitable that the largest individuals are included and that small or embryo snails are few or lacking. If the largest 10% is sorted from the rest on the basis of length of body whorl, and a series of adults (e.g., 25) is chosen at random from these large snails, it then is evident that there is not a great amount of variability in ensuing conchological measurements. For example, the mean length of body whorl of large *S. libertina* from Shimoda was 19.2 mm, with a standard error of the mean of 0.33 mm (Table 2). I subsequently found that this mean length did not change significantly in this population for a 2-year period. I consider the largest 10% of a population to represent the mature condition of the population.

Adult-hood may be defined as that age and size where reproduction is achieved. The problem of using this criterion is that embryos are often found in females which are less than ¾ grown. If adults are characterized by these snails that are in the reproductive state, then the various traits such as spire angle and ribs, etc., must be analyzed in terms of different size classes, and the description for a species then becomes very cumbersome. Additionally, it is of little help to consider as mature snails only those which have stopped growing. There are no objective methods for determining if the snail has stopped growing by simply observing the shell. No varix is formed at the end of growth, as in some hydrobiid snails, although an indication of the slowing-down of growth is often seen in the change in sculpture or color pattern on the body whorl near the outer lip. Ribs which are regularly positioned on the apical whorls, may become irregularly positioned on the body whorl, or they may fade out altogether. Spiral cords may fade out on the body whorl, which often becomes thickened and wrinkled. However, these sculpture conditions resulting from slower growth may persist for a whorl or more (seen in *Semisulcospira reticulata*), which involves a considerable increase in shell length during the period.

No post reproductive stage has been observed in any of the species studied. The largest adult females have had full complements of embryos in their brood chambers. Because of this, and the above factors, it is reasonable to discuss the characteristics of adult and mature snails in terms of the largest 10% of the population. In the absence of knowledge on population structure, this arbitrary level allows for an objective comparison of different populations. It is particularly valid when 300 or more specimens are easily collected from a large and prominent population, which is usually the case. Only *S. habei yamaguchi* and *S. multigranosa* of this study appeared to be rare and diffuclt to locate and collect.

Species groups, characters and the discrimination of taxa.

It is useful to create species groups or complexes within *Semisulcospira* based on the primary characters given in Table 41. The validity of considering the *S. libertina* and *S. niponica* complexes genetically distinct depends upon (1) further studies in cytogenetics as well as future investigations in biochemistry, and (2) further morphological and cytological data from numerous other populations of *Semisulcospira*, including named taxa from Japan not included in this study.

The *Semisulcospira libertina* group is characterized by taxa with a chromosome number of n=18 or 20, adult shells with 7 or more basal cords, and numerous young in the female brood pouch (generally 100 or more). *S. libertina* and *S. reiniana* seem to occupy central positions in the complex, while *S. kurodai* is considered marginal and transitional. *S. kurodai* has a chromosome number of n=18, although the numbers of basal cords ($\bar{X}$ of 5.1) and embryos per brood pouch ($\bar{X}$ of 35.5) are intermediate between the 2 species groups (observe Text Figs. 11 and 12).

The *Semisulcospira niponica* complex is comprized by taxa with chromosome numbers ranging from n=7 to n=14. The adult shells have 2 to 6 basal cords, and there are few embryos per brood pouch (less than 50, usually 35 or less). Species of this complex are endemic to Lake Biwa or its tributaries, and include *S. niponica*, *S. decipiens*, *S. reticulata*, *S. multigranosa*, *S. habei habei*, *S. habei yamaguchi* and *S. nakasekoae*.

Semisulcospira niponica is distinct by both its shell characters and its ecology. It belongs to the "rupicolous" association described for Lake Biwa by Annandale (1922), i.e., those animals habitually clinging to rocks or stones of either shallow or deep water. Typical *S. reticulata* is also distinct in regard to shell characters of adults and embryos, and it is characterized ecologically by being a deep-water (but not profundal) species. *S. decipiens*, *S. multigranosa* and *S. habei yamaguchi* are sympatric; they have an immediate association in their habitats. In the broader sense, 5 species of this complex (excluding *S. nakasekoae* and *S. habei habei*) are sympatric in Lake Biwa, i.e., they live in the same general area. *S. decipiens*, *S. multigranosa* and *S. habei yamaguchi* are sibling species, in that they are difficult to distinguish on shell characters (e.g., they have overlapping ranges of spire angles and rib numbers, and variable strength of nodes on the ribs). It was only when distinct chromosomal differences were found that they could be clearly separated and the limits of shell sculpture variability established. But once they were clearly separated by cytological differences, other specific features became evident. My results here agree with Simpson's (1962) statement that "there are extremely few examples in which species called sibling did not prove to be anatomically distinct when studied more carefully and with simultaneous consideration of all available anatomical characters."

Chromosome number and karyotype data (Burch & Davis, 1967; Burch, 1968) serve to distinguish most of the taxa discussed in this paper. For instance, Burch (1968) stated that *Semisulcospira kurodai* differed from *S. libertina* in that the former had relatively large numbers of acrocentric and subterminally constricted chromosomes, while the latter had mostly metacentric chromosomes (2n=36 in both). It is fortunate that cytological data are so useful in distinguishing taxa in this difficult group where conchological characters are notoriously variable. However, certain limitations and problems relating to the cytological data should be considered when using this information to characterize lower taxa such as species. For example, chromosome number variability probably due to supernumery elements, is present in certain taxa discussed here, and it is not known if taxa separated by a small difference in

chromosome number can hybridize. Hybridization, however, is probably not likely when different karyotypes are involved, e.g., a snail with metacentric small chromosomes probably cannot produce offspring with a snail having acrocentric large chromosomes. A difference in chromosome number would further complicate potential for pairing of these structurally different elements. Further, two or more distinct species may have the same chromosome number and seemingly similar karyotypes. It is important therefore to establish criteria in addition to cytological data for distinguishing between lower taxa.

When taxa are compared in terms of classical conchological features it is evident that inter-population variability exists where ranges of values about the mean overlap very much (e.g., spire angle of adult shells, Text Fig. 18). Comparisons of taxa in terms of many of these shell features thus involves statistical treatment. Because of this variability it is evident that a given species has relatively few traits which clearly serve to set it off from other species (i.e., presence of features with non-overlapping variation). Traits associated with adult shell size are particularly variable and unreliable in discriminating between species, e.g., total shell length, shell width, length of the body whorl and (in part) spire angle. Because of the usual erosion of the shell, counts of whorl number are of little use. As has been discussed above (p 270) the presence or absence of ribs does not serve to distinguish between species, and ribbing may vary from population to population, particularly in *Semisulcospira libertina*.

Potentially reliable criterea for defining species are found in the primary traits (Table 41), coupled with embryo size, morphology and intrabrood pouch developmental patterns. Developmental sequences of the embryos help in understanding the ontogeny of shell features basic to the species. The ontogeny of ribs in *Semisulcospira nakasekoae* (p 235) and *S. kurodai* (p 235) are cases in point; also, the globose condition of the embryos of *S. nakasekoae* at 3.5 whorls is correlated with the great spire angle of the adult shells (p 235). Finally, added to the above features are selected traits associated with the ribs on the adult shell, such as numbers of nodes and ribs (especially pertinent in the *Semisulcospira niponica* complex).

The full use of embryo characters for providing reliable traits for species discrimination is dependent on future results of studies involving many more populations of a species such as *Semisulcospira libertina*. For example, embryos of topotypes of *S. libertina* do not have nodes or ribs, according to my initial investigations in 1965. Later, in 1967 I found 1 of 1000 embryos from females of the Shimoda population which had nodes on the shoulder of the body whorl of a 3 whorl embryo. Recently (Davis; 1967b, 1969) I studied topotypes of so-called *S. trachea* (Westerlund) from Ashino Lake, Hakone Mountains, Kanagawa Prefecture, Japan, and as a result of the investigation placed this taxon in synonymy under *S. libertina*. The decision was based on data involving cytology, morphological studies similar to those of this paper, immunology and electrophoresis of proteins from foot muscle extract. The embryos of *"S. trachea"* uniformly had nodes on the shoulder of the whorls. The type of question which arises and which future investigations should answer is: Does some population exist where the embryos have distinct ribs yet all other features fit the present concept of *S. libertina* presented here and in Davis (1967b, 1969)?

Endemism of *Semisulcospira* in Lake Biwa.

With the exception of *Semisulcospira libertina* and *S. kurodai*, all the species which are thus far considered to be distinct valid species come from the immediate Lake Biwa area. *S. reiniana* has been observed living along the shores of the lake. The question natu-

rally asked is: Why are so many species found in such close association? Such speciation as evidenced in Lake Biwa can be understood in part, when one considers the unique features of this lake. As summed up by Annandale (1922), Lake Biwa lies towards the northern fringe of the extension of the Oriental region where extensions of southern faunas characteristic of South China are found. The abyssal fauna of the lake appears to be a relic of a period when the Palearctic fauna extended farther south than it does currently. These ideas are reiterated by Ueno (1937) and again by Horie (1961). Kawamura (1918) gives evidence for a wide belt of overlap in faunas from the Oriental and Palearctic regions (based on fishes and a few invertebrates). Most of Korea is in this belt, as are Kyushu Island, and Honshu Island to a point north of Tokyo.

Lake Biwa is the largest lake in Japan. It has a maximum depth of 104 meters (Horie, 1962) with an area of 674.4 km^2. Horie (1961, 1962) reviews other morphometric features of the lake, and the antiquity of Lake Biwa is discussed by Horie (1961) in light of the available literature. The lake dates back to the Tertiary Period, and its basal deposits are probably pre-Pliocene in origin. In these ancient times, the lake was 60 to 290 meters above its present level, and it extended considerably south (50 km or more) of its present position. With downwarping of the Tertiary peneplain accompained by faulting, the lake level was considerably lowered and the basin has been relatively isolated by barriers for about 1 million years without marked geological disturbances (Horie, 1961).

Endemicity in the lake was discussed by many writers (e.g., see Annandale, 1922; Horie, 1961). As stated by Matsushita (1963), "frequent land connections with the East Asian continent probably from some age of the Mesozoic to the Pleistocene" were characteristic for the geology of Japan.

Semisulcospira is a part of the Oriental fauna characteristically found in abundance in the belt of regional overlap discussed by Kawamura (1918), i.e., throughout Honshu, Kyushu, Korea, and the Ryukyu Islands, as well as Taiwan (Formosa) and mainland China. Quite possibly, *Semisulcospira* invaded Japan from East Asia several times with 1 or 2 representatives of different genetic stock (i.e., the *S. niponica* and *S. libertina* groups?).

Several conditions for speciation have been present within the lake: an immense lacustrine volume divided into numerous niches in the accompanying drainage systems, and a long period of time. Endemism and sympatry have probably resulted because of the drop in lake level with subsequent appearance of elevated barriers preventing immigration or emigration. The shrinking lake forced the association of numerous forms within the limits of the present lake basin and the present single drainage system of the Setagawa River. The drop in lake level possibly resulted in other populations perishing or being excluded to other drainage systems. One such example of possible exclusion is *Semisulcospira kurodai*.

A million years of relative geological stability probably allowed further speciation or incipient speciation such as evidenced by *Semisulcospira habei habei* and *S. habei yamaguchi*. The former is found within the Setagawa River system and the latter is spread throughout Lake Biwa and is sympatric with populations of other species of *Semisulcospira* living in the lake.

The *Semisulcospira libertina* complex is associated with a genetic potential permitting invasion of the Palearctic region of Japan, i.e., northern Honshu and southern Hokkaido. Members of this complex are well represented throughout the belt of regional faunal overlap, as well as north of that belt.

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APPENDIX 1

Statistics of embryo shell dimensions for each whorl stage for species of Japanese *Semisulcospira*

Species	No. specimens	Embryo whorl stage	Shell feature measured	Statistic		
				$\bar{X}$	S	Se
S. libertina		2.0	L	0.95	0.048	0.010
Shimoda	25		W	0.74	0.055	0.011
			LBW	0.78	0.069	0.014
	24	2.5	L	1.16	0.301	0.061
			W	0.84	0.058	0.012
			LBW	0.90	0.067	0.014
	16	3.0	L	1.35	0.094	0.023
			W	0.93	0.050	0.013
			LBW	0.99	0.059	0.015
Amami-oshima	25	2.0	L	0.92	0.081	0.016
			W	0.74	0.060	0.012
			LBW	0.78	0.075	0.015
	25	2.5	L	1.18	0.077	0.015
			W	0.84	0.087	0.017
			LBW	0.96	0.062	0.012
	4	3.0	L	1.39	0.078	0.039
			W	0.99	0.026	0.013
			LBW	1.08	0.035	0.018
S. reiniana	25	2.0	L	0.93	0.102	0.020
			W	0.78	0.078	0.016
			LBW	0.79	0.108	0.022
	25	2.5	L	1.28	0.116	0.023
			W	0.97	0.097	0.019
			LBW	1.03	0.082	0.016
	25	3.0	L	1.64	0.119	0.024
			W	1.19	0.086	0.017
			LBW	1.21	0.104	0.021

Appendix 1 (cont'd)

Species	No. specimens	Embryo whorl stage	Shell feature measured	Statistic		
				$\bar{X}$	S	Se
S. reiniana (cont'd)	23	3.5	L	1.89	0.118	0.025
			W	1.29	0.072	0.015
			LBW	1.37	0.088	0.018
S. kurodai	25	2.0	L	0.96	0.073	0.015
			W	0.83	0.083	0.017
			LBW	0.81	0.086	0.017
	25	2.5	L	1.25	0.135	0.027
			W	0.99	0.114	0.023
			LBW	1.02	0.149	0.030
	25	3.0	L	1.62	0.110	0.022
			W	1.15	0.067	0.013
			LBW	1.23	0.110	0.022
S. nakasekoae	25	2.0	L	1.14	0.093	0.019
			W	1.05	0.089	0.018
			LBW	1.05	0.088	0.018
	25	2.5	L	1.59	0.197	0.039
			W	1.38	0.148	0.030
			LBW	1.42	0.179	0.036
	25	3.0	L	2.28	0.274	0.055
	24		W	1.86	0.286	0.058
	25		LBW	2.01	0.247	0.049
	9	3.5	L	2.99	0.344	0.115
			W	2.40	0.210	0.070
			LBW	2.57	0.278	0.093
S. habei	17	2.0	L	1.21	0.157	0.050
			W	1.11	0.136	0.044
			LBW	1.09	0.131	0.042
	25	2.5	L	1.63	0.198	0.040
			W	1.44	0.141	0.028
			LBW	1.43	0.156	0.031

Appendix 1 (cont'd)

Species	No. specimens	Embryo whorl stage	Shell feature measured	Statistic		
				$\bar{X}$	S	Se
S. habei (cont'd)	25	3.0	L	2.23	0.212	0.042
			W	1.79	0.139	0.028
			LBW	1.86	0.158	0.032
	25	3.5	L	2.59	0.162	0.032
			W	2.00	0.123	0.025
			LBW	2.09	0.189	0.038
S. habei yamaguchi	10	2.0	L	1.06	0.088	0.028
			W	0.96	0.079	0.025
			LBW	0.96	0.066	0.021
	5	2.5	L	1.35	0.099	0.044
			W	1.15	0.055	0.025
			LBW	1.16	0.073	0.033
	6	3.0	L	1.78	0.128	0.052
			W	1.35	0.078	0.032
			LBW	1.47	0.095	0.039
	7	3.5	L	2.15	0.161	0.061
			W	1.56	0.181	0.068
			LBW	1.68	0.051	0.019
S. niponica	25	2.0	L	1.14	0.136	0.027
			W	1.08	0.104	0.021
			LBW	1.04	0.129	0.026
	25	2.5	L	1.59	0.200	0.040
			W	1.44	0.177	0.035
			LBW	1.42	0.170	0.034
	25	3.0	L	2.35	0.156	0.031
			W	1.87	0.121	0.024
			LBW	2.01	0.146	0.029
	20	3.5	L	2.83	0.222	0.050
			W	2.11	0.149	0.033
			LBW	2.30	0.183	0.041

Appendix 1 (cont'd)

Species	No. specimens	Embryo whorl stage	Shell feature measured	Statistic		
				$\bar{X}$	S	Se
S. decipiens	25		L	1.35	0.115	0.023
	25	2.5	W	1.08	0.256	0.051
	9		LBW	1.30	0.074	0.025
	25		L	1.83	0.173	0.035
	25	3.0	W	1.28	0.107	0.021
	9		LBW	1.53	0.103	0.034
	25		L	2.23	0.202	0.040
	25	3.5	W	1.45	0.119	0.024
	9		LBW	1.85	0.106	0.035
S. reticulata			L	1.24	0.307	0.085
Station 2	13	2.0	W	1.19	0.160	0.044
Shell type 1			LBW	1.18	0.150	0.042
			L	1.82	0.257	0.054
	20	2.5	W	1.64	0.024	0.051
			LBW	1.59	0.029	0.061
			L	2.59	0.36	0.108
	11	3.0	W	2.07	0.21	0.063
			LBW	2.24	0.27	0.081
			L	3.60	0.51	0.128
	16	3.5	W	2.61	0.367	0.093
			LBW	2.98	0.688	0.173
			L	4.71	0.39	0.13
	9	4.0	W	3.12	0.32	0.107
			LBW	3.64	0.39	0.13
			L	5.65	0.37	0.140
	7	4.5	W	3.57	0.228	0.087
			LBW	4.27	0.27	0.102
S. multigranosa			L	1.52	0.224	0.085
	7	2.5	W	1.25	0.162	0.061
			LBW	1.33	0.170	0.064

Appendix 1 (cont'd)

Species	No. specimens	Embryo whorl stage	Shell feature measured	Statistic		
				$\bar{X}$	S	Se
S. multigranosa (cont'd)	6	3.0	L	2.22	0.165	0.067
			W	1.51	0.252	0.103
			LBW	1.85	0.161	0.066
	11	3.5	L	2.77	0.341	0.103
			W	1.84	0.137	0.041
			LBW	2.28	0.242	0.073
	4	4.0	L	3.53	2.94 - 4.18 (range)	
			W	2.09	1.88 - 2.31 (range)	
			LBW	2.85	2.25 - 3.31 (range)	
	10	4.5	L	4.67	0.227	0.072
			W	2.54	0.178	0.056
			LBW	3.66	0.193	0.061

L = length
W = width
LBW = length of the body whorl

$\bar{X}$ = mean
S = standard deviation
Se = standard error of the mean

APPENDIX 2

Embryo shell features of Japanese *Semisulcospira* measured or counted

Species	Statistics	Apical diameter		Number of ribs
		Apical whorl (mm)	Tip of apical whorl (mm)	
S. libertina				
Shimoda	$\bar{X}$	0.51	0.19	0
	S	0.03	0.018	-
	Se	0.006	0.004	-
	No.	25	25	-
Amami-oshima	$\bar{X}$	0.47	0.18	0
	S	0.03	0.02	-
	Se	0.006	0.004	-
	No.	25	25	-

Appendix 2 (cont'd)

Species	Statistics	Apical diameter		Number of ribs
		Apical whorl (mm)	Tip of apical whorl (mm)	
S. reiniana	$\bar{X}$	0.46	0.20	16.0
	S	0.05	0.05	1.60
	Se	0.007	0.007	0.32
	No.	49	49	25
S. kurodai	$\bar{X}$	0.46	0.16	15.0*
	S	0.039	0.027	2.26
	Se	0.005	0.004	0.54
	No.	51	51	17
S. nakasekoae	$\bar{X}$	0.44	0.17	14.0
	S	0.051	0.023	1.63
	Se	0.008	0.003	0.45
	No.	46	46	13
S. habei	$\bar{X}$	0.51	0.16	13.0
	S	0.066	0.018	1.60
	Se	0.008	0.002	0.17
	No.	68	57	88
S. habei yamaguchi	$\bar{X}$	0.47	0.16	14.0
	S	0.040	0.02	1.45
	Se	0.009	0.004	0.34
	No.	22	22	18
S. niponica	$\bar{X}$	0.47	0.16	13.0
	S	0.065	0.025	1.17
	Se	0.009	0.004	0.19
	No.	48	48	36
S. decipiens	$\bar{X}$	0.45	0.15	11.0
	S	0.028	0.019	0.85
	Se	0.005	0.004	0.15
	No.	31	31	31

Appendix 2 (cont'd)

Species	Statistics	Apical diameter		Number of ribs
		Apical whorl (mm)	Tip of apical whorl (mm)	
S. reticulata	$\bar{X}$	0.58	0.17	14.0
Station 2	S	0.077	0.031	1.43
	Se	0.009	0.003	0.24
	No.	61	61	35
S. multigranosa	$\bar{X}$	0.51	0.016	10 ± 2
Station 3	S	0.060	0.03	-
	Se	0.01	0.006	-
	No.	24	23	-

$\bar{X}$ = mean
S = standard deviation
Se = standard error of the mean
No. = number of observations

*generally there are only 15 ($\bar{X}$) nodes which are rarely elongated into ribs.

RÉSUMÉ

UNE ÉTUDE TAXONOMIQUE SUR QUELQUES ESPÈCES DE *SEMISULCOSPIRA* DU JAPON (MESOGASTROPODA: PLEUROCERIDAE)

G. M. Davis

Le but de cette étude est d'établir les concepts taxonomiques de base pour 10 taxa du groupe-espèce concernant les mollusques dulcicoles du genre *Semisulcospira*. Plus de 30 espèces et sous-espèces de ce genre ont été décrites du Japon, y compris les îles Ryuku et Ogasawara. On a obtenu pour la présente étude, les topotypes des espèces précédemment décrites les plus remarquables. Deux taxa sont décrits comme nouveaux, *Semisulcospira habei habei* et *S. habei yamaguchi*.

Les topotypes ont été analysés dans la proportion de plus de 10% de chaque population. Des critères ont été établis sur la morphologie de la coquille adulte, de la coquille embryonnaire et de la poche incubatrice. Les critères ont été analysés et présentés de façon à permettre au lecteur de comprendre les variations naturelles du paramètre mesuré ou compté. On a établi une corrélation avec les études cytologiques de Burch & Davis (1967) et Burch (1968), en vue d'établir des critères spécifiques.

Les taxa ont été réduits à deux groupes-espèces, le groupe de *Semisulcospira libertina* et le groupe *S. niponica*. Le premier est caractérisé par un nombre de chromosomes n=18 ou 20, des coquilles adultes ayant des bourrelets basaux au nombre de 7 ou plus, et par les nombreux jeunes (100 ou plus) se trouvant dans la poche incubatrice (oviducte modifié) de la femelle. *S. libertina* et *S. reiniana* sont les principales espèces de ce complexe. *S. kurodai* est placé dans ce groupe à cause de nombre de chromosomes de ce taxon, qui est n=18; cependant, cette espèce est considérée comme intermédiaire entre les 2 groupes-espèces du fait que, en moyenne, la coquille adulte a 5,1 bourrelets basaux et qu'il y a 35,5 ± 15,4 embryons par poche incubatrice chez la femelle.

Le groupe espèce *Semisulcospira niponica* est caractérisé par un faible nombre de chromosomes, n=7 à 14; par des coquilles adultes ayant 2 à 6 bourrelets basaux et par un faible nombre d'embryons par poche incubatrice (une moyenne de 25,2 ± 9,8 maximum à 5,2 ± 3,4 minimum, selon les espèces). Les taxa compris dans ce groupe sont des endémiques du lac Biwa et de son bassin hydrologique; ce sont: *S. niponica, S. decipiens, S. reticulata, S. habei habei, S. habei yamaguchi et S. nakasekoae.*

Une clé des espèces est fournie pour faciliter l'identification. L'utilité des critères utilisés dans la description des taxa est discutée. Les caractères fondamentaux pour définir les espèces sont: le nombre de chromosomes, le nombre de bourrelets basaux de la coquille adulte, le nombre d'embryons portés par la femelle, l'ontogénie de l'ornementation de la coquille, le nombre de stries et de nodosités sur la coquille adulte, la taille et la forme de l'embryon, les modes de croissance des embryons dans la poche incubatrice, le nombre de tours de spire atteint par l'embryon dans la femelle, la texture et la coloration de l'embryon.

Plusieurs caractères semblent être sujets à variation à l'intérieur d'une population. Ce sont la largeur de la coquille, l'angle de la spire, la longueur du dernier tour de spire, la microtexture de l'embryon, les mesures du tour apical, et la coloration de l'adulte. Dans le groupe-espèce *Semisulcospira libertina*, la présence ou l'absence de stries et la texture de l'embryon sont sujets à de telles variations. L'angle de spire, cependant, est utile pour la différenciation de plusieurs espèces. Le nombre de tours de spire et la longueur de la coquille adulte sont sous la dépendance du milieu.

Semisulcospira habei yamaguchi, S. decipiens et *S. multigranosa* sont des espèces morphologiquement semblables. De plus, *S. multigranosa* est polymorphe ayant des formes lisses et striées, et 3 types de coloration. Quand les résultats d'études cytologiques et de la morphologie embryonnaire sont mis en corrélation, il devient évident que ce sont des espèces distinctes. De nouveaux caractères de coquilles décelables ont alors été notés. Dans le lac Biwa, les espèces suivantes sont sympatriques: *S. habei yamaguchi, S. decipiens, S. multigranosa, S. reticulata* et *S. niponica.*

La majorité des espèces étudiées sont endémiques dans l'aire du lac Biwa, Préfecture de Shiga, Honshu. Plusieurs des conditions requises pour la spéciation sont présentes dans le lac Biwa. Le lac est ancien (Tertiaire) et a eu environ 1 million d'années de stabilité; il a un immense volume lacustre divisé en nombreuses niches. Endémisme et sympatrisme résultent peut être de ce que, lorsque le niveau du lac s'abaisse, des barrières apparaissent qui empêchent l'émigration et l'immigration. La rétraction du lac oblige les associations de nombreux organismes à demeurer à l'intérieur des limites du bassin hydrographique du lac et du seul système de drainage de la rivière Setagawa. Les autres populations ont été exclues du lac ou ont péri. *Semisulcospira kurodai* peut être un exemple d'une telle exclusion. Un million d'années de stabilité ont problement permis une nouvelle spéciation ou une speciation naissante, par ex. *S. habei habei* et *S. habei yamaguchi.*

RESUMEN

ESTUDIO TAXONOMICO DE ALGUNAS ESPECIES DE *SEMISULCOSPIRA* EN JAPON (MESOGASTROPODA: PLEUROCERIDAE)

G. M. Davis

El objeto de este trabajo es el de establecer conceptos taxonómicos básicos para 10 "grupos de especies" diferentes, del género de gastropodos dulceacuícolas *Semisulcospira.* Más de 30 especies y subespecies de este este género han sido denominadas para el Japón, incluyendo las islas Ryukyu y Ogasawara. Para el estudio se obtuvieron topotipos de las especies previamente descriptas más prominentes. Dos se describen aqui como nuevas: *Semisulcospira habei habei* y *S. habei yamaguchi.*

Los topotipos fueron analizados en términos del 10% de los más grandes en cada población. Se reunieron todos los datos sobre la morfología de la concha adulta,

caracteres conchológicos embrionarios y el desarrollo de los embriones incubados en el saco marsupial. Los datos se analizan y se presentan en tal forma que puedan permitir al lector estimar la variación natural en los parámetros medidos y contados; tambien se correlacionaron con los descubrimientos citológicos de Burch & Davis (1967) y Burch (1968), para establecer conceptos de las especies.

Los taxa se ubicaron en 2 grupos, el de *Semisulcospira libertina*, y el de *S. niponica*. El primer grupo se caracteriza por tener un número cromosomático de n=18 o 20, conchas adultas con 7 o mas cordones basales, y gran cantidad (100 o mas) de juveniles en el saco marsupial (oviducto paleal modificado). *S. libertina* y *S. reiniana* son las especies principales del complejo. *S. kurodai* se coloca en este primer grupo porque su número cromosomático es n=18; sin embargo, la especie se considera como de transición entre los 2 grupos de especies, ya que el adulto tiene por término medio 5,1 cordones basales y 35,5 ± 15,4 embriones en el saco marsupial de cada hembra.

El grupo de *Semisulcospira niponica* está caracterizado por el número cromosomático n=7 a 14, conchas adultas con 2 a 6 cuerdas basales, y pocos embriones en la marsupia (25,2 ± 9,8 maximo, a 5,2 ± 3,4 mínimo, dependiendo de la especie). Los taxa incluídos, en este grupo endémico en el Lago Biwa y su desague, son: *S. niponica*, *S. decipiens*, *S. reticulata*, *S. habei habei*, *S. habei yamaguchi* y *S. nakasekoae*.

Se da una clave para ayudar a la identificación de las especies. Se discute la utilidad de los rasgos usados en las descripciones. Caracteres de importancia básica para definir las especies son: número cromosomático, número de cuerdas basales de la concha adulta, cantidad de embriones llevados por la hembra, ontogenia de la escultura conchológica, número de costulaciones y nudos en la concha adulta, forma y tamaño del embrión, patrón de crecimiento de los embriones en el saco marsupial, tamaño que alcanza el anfracto embrionario dentro del saco, escultura embrional y diseño de color.

Algunos rasgos parecen estar particularmente sujetos a variación intra-poblacional. Estoa son, el ancho de la concha adulta, ángulo espiral, longitud del último anfracto, microescultura embrional, medidas del anfracto apical y diseños de color en el adulto. En el grupo de especies de *S. libertina* la presencia o ausencia de costillas y escultura embrional están sujetas a mucha varación. El ángulo apical es, sin embargo, útil para la diferenciación entre varias especies. El número de anfractos y longitud de la concha adulta están sometidos al control del ambiente.

Semiculcospira habei yamaguchi, *S. decipiens*, y *S. multigranosa* son especies gemelas (sibling). *S. multigranosa* es polimórfica por tener tanto formas lisas como costuladas, y tres patrones de color distintos. Cuando se correlacionaron los datos de estudios citológicos con la morfología embrional, se puso en evidencia las diferencias entre esas especies. Se distinguieron entonces otros caracteres conchológicos. En el lago Biwa las siguientes especies son simpátricas: *S. habei yamaguchi*, *S. decipiens*, *S. multigranosa*, *S. reticulata* y *S. niponica*. La mayoría de las especies aquí estudiadas son endémicas en el área del lago Biwa, Prefectura de Shiga, Honshu. Varias condiciones para la especificación estan presentes en el lago. El lago es antiguo (terciario) y se ha estabilizado hace un millon de años; tiene un volúmen lacustre inmenso dividido en numerosos nichos. Endemismo y simpatría comenzaron quizá cuando el nivel del lago bajó, con la subsecuente elevación de barreras que no permitieron inmigración o emigración. El estrechamiento del lago forzó la asociación de numerosos organismos dentro de los límites del lago corriente y el único sistema de desague del lago Setagawa. Otras poblaciones fueron excluídas del lago o perecieron. *Semisulcospira kurodi* puede ser un ejemplo de tal exclusión. Un millon de años de estabilidad permitió probablemente mayor o incipiente especiación, por ejemplo *S. habei habei* y *S. habei yamaguchi*.

АБСТРАКТ

ТАКСОНОМИЧЕСКОЕ ИЗУЧЕНИЕ НЕКОТОРЫХ ВИДОВ *SEMISULCOSPIRA* (MESOGASTROPODA, PLEUROCERIDAE) ЯПОНИИ

Г.М.ДЕВИС

В работе рассматриваются некоторые основные концепции для установления систематического положения 10 различных групп видов пресноводных моллюсков из рода *Semisulcospira*. Более 30 видов и подвидов этого рода было найдено в Японии, включая острова Рюкю и Огасавара. Во время исследования были найдены топотипы наиболее известных из ранее описанных видов. Описываются два новых таксона: *Semisulcospira habei habei* и *S. habei yamaguchi*. Топотипы анализировались в количестве 10% от каждой популяции. Собраны данные по морфологии раковины взрослых форм и эмбрионов и по развитию эмбриональной раковины внутри выводковой камеры. Полученные данные анализировались и представлены в статье для получения естественных вариаций измеренных или подсчитанных параметров. Эти данные согласованы с цитологическими открытиями, полученными Бёрчем и Девисом (1967) и Бергом (1968), чтобы установить видовую специфику.

Таксоны распадаются на 2 группы: *Semisulcospira libertina* и *S. niponica*. Первая группа характеризуется наличием числа хромосом n=18 или 20, семью или более базальными ребрами (cords) на взрослой раковине; молодь в выводковой сумке самки (модифицированном мантийном яйцеводе) - многочисленна (100 или более экземпляров).

Основные виды этой группы *S. libertina* и *S. reiniana*. *S. kurodai* отнесен к этой группе, т.к. число хромосом у него равно 18; однако этот вид рассматривается как переходный между двумя группами видов, поскольку взрослая раковина у него имеет в среднем 5,1 базальных ребер, а количество эмбрионов в выводковой сумке каждой самки составляет 35,5± 15,4.

Группа видов *Semisulcospira niponica* характеризуется меньшим числом хромосом n=от 7 до 14; взрослая раковина имеет от 2 до 6 базальных cords, а количество эмбрионов в выводковой сумке каждой самки очень мало (в среднем наиболвщее число 25,2 ± 9,8, минимум, 5,2 ± 3,4 что зависит от вида). Виды, отнесенные к этой группе являются эндемиками озера Бива и его притоков Это - *S. niponica, S. decipiens, S. reticulata, S. habei habei, S. habei yamaguchi* и *S. nakasekoae*.

Имеется ключ для определения видов. В работе обсуждаются валидность тех или иных признаков, употребляющихся при видовом описании. Основными признаками считаются: число хромосом, количество базальных ребер на взрослой раковине, количество эмбрионов в выводковых сумках, онтогения скульптуры раковины, количество и форма эмбрионов, особенности роста эмбрионов в выводковой камере и размер оборотов раковины находящихся в ней эмбрионов, их скульпт у ра и окраска. Некоторые признаки видимо подвержены межвидовой изменчивости. Это ширина взрослой раковины, угол макушки, длина основного завитка, микроскульптура эмбрионов, изменения апикального оборота и окраска взрослых форм. У группы *Semisulcospira libertina* ь наличии или отсутствии ребер и скульптуры у эмбрионов имеются также вариации. Угол вершины макушки может служить для различия между некоторыми видами. Количество оборотов и длина взрослой раковины зависит от условий среды.

Semisulcospira habei yamaguchi, S. decipiens и *S. multigranosa* являются родственными видами. Последний очень полиморфный вид, имеющий гладкие и ребристые морфы, 3 цветных пятна. После сравнения цитологических данных и морфологии эмбрионов стало очевидным, что это разные виды. В дальнейшем были получены и различия в раковинах. В озере Бива обитают вместе *S. habei yamaguchi, S. decipiens, S. multigranosa, S. reticulata* и *S. niponica*.

Большая часть изученных видов эндемичны для области озера Бива, префектура Шига, Хонсю. Некоторые условия озера способствуют видообразованию. Озеро весрма древнее (третичное) и мало менялось втечение последнего млн. лет; оно имеет огромный объем, распадющийся на многочисленные ниши. Эндемизм и симпатрия возможно возникли, когда уровень озера понижался и возникали барьеры и поднятия, затруднявшие иммиграцию или эмиграцию. Усыхающее озеро приводило к образованию многих групп организмов в бассейне проточного озера с единственным стоком ввиде реки Сетагава. Остальные популяции населения исчезли из озера или погибли. ***Semisulcospira kurodai*** может служить примером такого исчезновения. Миллионы лет стабильности возможно привели к дальнейшему видообразованию или к его начальным стадиям, как в случае ***S. habei habei*** и ***S. habei yamaguchi***.

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STUDIES ON THE LOCOMOTOR ACTIVITY OF THE SLUG *ARION ATER* (LINNAEUS)

I. HUMIDITY, TEMPERATURE AND LIGHT REACTIONS

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ABSTRACT

The reactions of the slug *Arion ater* (L.) to humidity, temperature and light were studied. The methods used employed a simple aktograph apparatus for recording locomotor activity. These demonstrated that adult *A. ater* are able to distinguish between high and low humidities, and generally show a preference for high humidity.

In the laboratory temperature changes have no effect on the timing of locomotor activity, and it is concluded that field activity is not controlled by the natural temperature cycles. *Arion ater* readily synchronise their activity with 24 hr. cycles of artificial light, and consequently it is suggested that natural light is the most important environmental factor in the control of field activity.

Slugs are nocturnal animals and appear from their cover of debris or holes in the ground soon after sunset. There is some evidence that each species has its own time of maximum nocturnal activity, and that during the summer the time of onset of activity is more closely related to the time of sunset than in the winter (Barnes & Weil, 1944). The active phase is divided into times of feeding, crawling, resting and copulating (Newell, 1966), and as they move some species, e.g. *Limax maximus* (Taylor, 1907), and *Agriolimax reticulatus* (Newell, 1966) describe a figure '8', and often return to the cover from which they appeared. Most slugs return to cover several hours before sunrise, but occasionally during dull and showery weather the active phase is extended for several hours after sunrise. These conditions also may advance the time of the onset of activity before sunset.

In common with other slugs, *Arion ater* remain under cover in the daytime with the tentacles withdrawn in the resting position. They become active soon after sunset, and exhibit the same types of activity as observed by Newell (1966) before returning to cover generally several hours before dawn. It is interesting to note that they often return to the cover from which they first appear by describing either simple or complex loops which do not cross over to form a figure '8'.

There are several factors which could possibly control the timing of the start of the active phase in slugs. Dainton (1954) found that temperature was the most important factor in the control of the activity of *Agriolimax reticulatus*. Temperature falls of as little as 0.1°C per hour below 21°C, and small increases above this temperature brought about activity, and so her conclusion was that in the field slugs were active

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after showers and at night because of the accompanying falls in temperature. Dainton also investigated other climatic factors and found that although an increase in light intensity initially caused a burst of activity, natural light cycles did not control the timing of the active phase. Likewise changes in atmospheric humidity did not stimulate activity. Karlin (1961) also considered that fluctuating temperature was the major factor promoting activity of slugs, and diminishing light apparently did not have any initiating effect on activity. In contrast, Newell (1968) showed a closer connection between the time of the active phase and the natural light cycles than with temperature cycles. His conclusion was that the fall in light intensity at sunset, rather than the lowering of temperature, initiated activity. None of these authors indicated any evidence of a lasting internal rhythm in slugs.

The aim of the work reported here was to analyse the field locomotor activity patterns of *Arion ater* by a series of laboratory experiments which were carried out at Cardiff, Wales, between June and October, 1965 and 1966. Experiments relating to the reactions of *A. ater* to humidity, temperature, and light are described here, and an account of the work on the endogenous control of the timing of activity is reported in a subsequent paper (Lewis, 1969). The experiments are not necessarily described in the order in which they were carried out, and so some experiments described in this paper were designed with a knowledge of the results of the experiments in the second paper.

MATERIALS AND APPARATUS

Locally collected adult *Arion ater* were studied throughout these experiments, and freshly collected slugs, or slugs which had been kept for a short time out of doors, were used whenever appropriate. The locomotor activity of the slugs was recorded in a simple circular aktograph (Fig. 1) which was 32 cm in diameter with a rim 2 cm high. The base was covered with damp sphagnum moss, and the chamber was enclosed with a plastic cover held over the rim with a cardboard ring. This cover was either completely black, or was made of half clear and half black material. The slugs were fed on carrot which was placed on the mid-line of the aktograph, and lasted for the duration of most of the experiments. The activity traces were drawn on a slow-moving kymograph drum, and are arranged in the text figures so that the consecutive daily patterns are displayed in sequence below each other. Each trace begins at 12:00 hours (noon), and, where necessary, the timing of the imposed entraining cycles are indicated.

The behaviour of slugs in this apparatus was shown to be normal by recording the activity of freshly collected slugs in the apparatus when subjected to natural conditions. The resulting activity patterns approximated well to the activity patterns observed in the field (Lewis, 1967).

I. HUMIDITY REACTIONS

The activity of slugs is influenced by the degree of hydration of the body (Howes & Wells, 1934; Wells, 1944), and as they are susceptible to desiccation in dry atmospheres, it was considered important to determine to what extent *Arion ater* was able to distinguish between dry and humid environments. Laboratory experiments were therefore carried out to study their reactions in a humidity-choice apparatus.

Method

The humidity-choice chamber (Fig. 2) was based on the aktograph apparatus described previously. A circular piece of Nylon net was held over the aktograph rim by a cardboard ring 4 cm high, and the 2 resulting chambers were enclosed with a polythene sheet held on with a

Fig. 1. Diagram of the aktograph (side view).

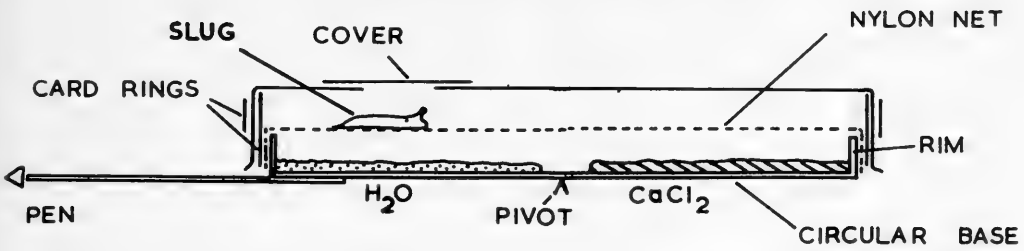

FIG. 2. Diagram of the humidity-choice chamber (side view).

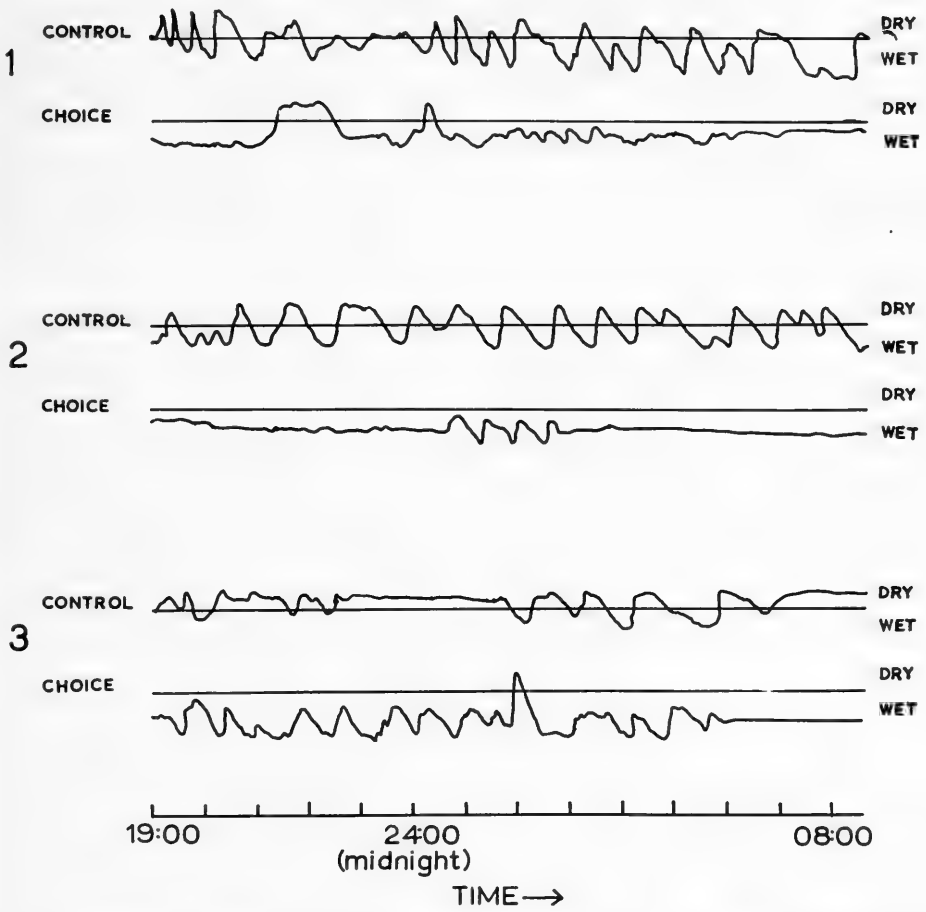

FIG. 3. Reactions to humidity by *Arion ater* during 3 trials. The wet and dry sides of the choice-chamber are indicated.

cardboard ring. The humidity-choice was produced in the lower chamber by damp cotton-wool and calcium chloride at either side of the mid-line. The method for measuring the relative humidity in the upper chamber employed glycerine solutions as described by Griffiths & Awberry (1926). The refractive index of glycerine solutions varies with the water content, and can be measured easily and accurately with an Abbé refractometer. Tables relating the refractive index of glycerine solutions with the relative humidity of air in equilibrium with the solutions are given by Griffiths & Awberry (1926). Consequently the refractive index of small volumes of glycerine solution which have equilibrated in air can be used as a measure of the relative humidity of the air. In the experiments centimetre squares of tissue paper were soaked in glycerine solution and left at various points in the chamber to equilibrate. The refractive indices were read by placing the glycerine paper directly in the refractometer and the relative humidities calculated from a graph derived from the figures of Griffiths & Awberry (1926). The relative humidity in either side of the upper chamber was about 30% and 100% at 10°C, with a steep gradient between the extremes. Two similar chambers were pivoted one above the other so that movements of each were recorded on 1 kymograph paper. The upper chamber acted as a control, and its base was covered entirely with damp cotton wool. One slug was placed in the upper half of each chamber, and their movements were recorded overnight.

Results

Three sets of the traces obtained are given in Fig. 3. Each slug was used in both a control and choice apparatus and then discarded. In a total of 11 trials all the slugs in the choice-chambers ended their activity in the wet side and generally remained there during the active phase, but occasionally slugs moved into the dry side where they stayed for up to 3 hours. The control slugs showed no preference for any part of the chamber. The conclusion was that *Arion ater* was able to distinguish between the wet and dry environments and generally showed a preference for the wet. No successful attempt was made to locate the humidity receptors, but increased head movements were observed at the boundary of steep gradients suggesting that the tentacles may bear the receptors. These possibly function through the lowering of the temperature of the tentacles resulting from the evaporation of water at low humidity.

II. TEMPERATURE REACTIONS

It is possible that the onset of slug activity in the evening is controlled in some way by the falling temperature at this time. The following experiments were therefore carried out to determine the effect of temperature changes on the timing of the locomotor activity of *Arion ater*.

Method

The locomotor activity was recorded in an aktograph covered in black material. The procedure adopted was to maintain slugs in the dark for several weeks at 10°C so that any endogenous activity pattern was weakened or lost. Individual slugs were enclosed in the aktograph and their activity was recorded first for 2 days in constant conditions, and afterwards when subjected to 24 hr. artificial temperature cycles for 5 days.

Results

The results of one of the 29 animals tested are recorded in Fig. 4 and show the effect of a 24 hr. cycle with a maximum temperature of 18°C and a minimum of 12°C, with transition periods of about 2 hours. There was no evidence that the temperature changes had any effect on the timing of activity in

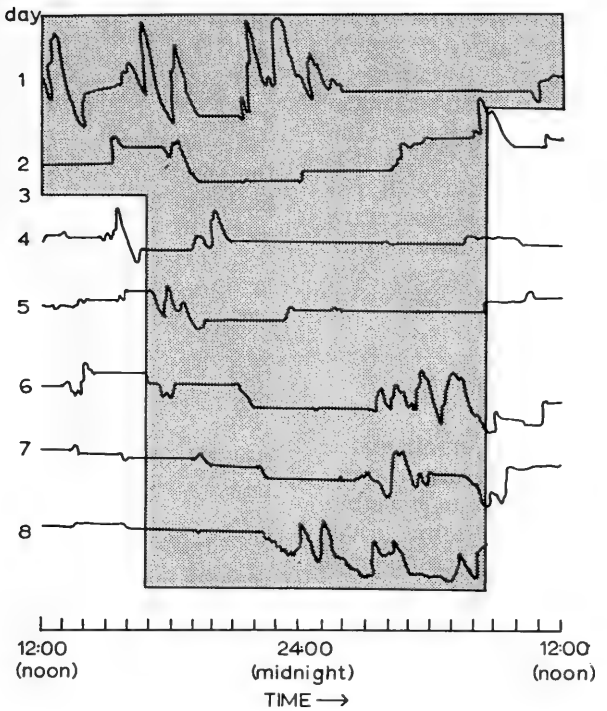

FIG. 4. Reactions to temperature by *Arion ater*, showing the effect of 24 hr. temperature cycles on locomotor activity. The cooler phases of the temperature cycles are shaded; the maximum temperature was 18° C, minimum 12° C.

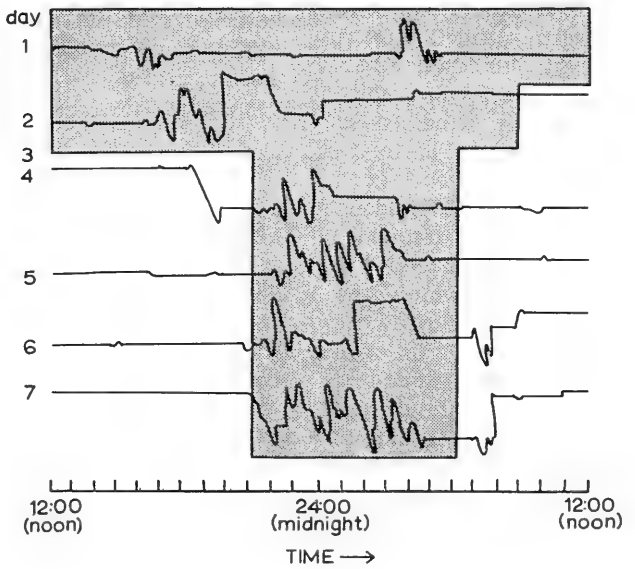

FIG. 5. The synchronisation of the locomotor activity of *Arion ater* with 24 hr. cycles of artificial light. The dark phases of the light cycles are shaded.

this experiment, nor were there evident effects when other larger or smaller changes were tried. It therefore seems unlikely that temperature changes had any effect on the timing of the locomotor activity of *Arion ater*, and it is probable that field activity is not controlled by the natural temperature cycles. This conclusion is notably different from Dainton's, but agrees with Newell's explanation of the control of activity in *Agriolimax reticulatus*.

III. LIGHT REACTIONS

As *Arion ater* generally remain under cover in the daytime and move into the open soon after sunset, it is possible that light is an important factor in the control of their behaviour. Experiments were therefore carried out to test their reactions in a light-choice chamber both in the daytime and at night, and to find the effect of changes in light intensity on the timing of activity.

1. Light-choice experiments in the daytime

Method

The first light-choice experiment was carried out in the daytime under natural light in a simple light-choice chamber. This consisted of a 60 x 24 cm white enamel tray, which was wiped over with a damp cloth and covered half with glass and half with glass and dark material. The covers did not produce a significant temperature difference. Eight *Arion ater* were placed in the light half of the chamber, which was positioned near a well lit window in the daytime. The number of slugs in each half, or in the middle were counted at frequent intervals for up to 2 hours. A control experiment, in which the whole of the chamber was covered in dark material, was carried out to assess the probability of slugs moving into the dark section by chance.

Results

The control animals moved around the chamber and often returned to the starting half, but their distribution at the end of the experiment was such that no more than 25% were in the other half. In the choice-chambers, however, only 10 of 48 slugs tested remained in the light half after an hour, and some of these 10 moved over after further exposure.

The conclusion of the experiment was that *Arion ater* showed a preference for shade rather than natural light, and if the same behaviour is shown in the field this could be why they remain under cover in the daytime.

2. Light-choice experiments at night

Method

The night light-choice experiment was devised to discover whether *Arion ater* were able to distinguish between small differences in light intensity at night, and to find out if there was a preference for high or low intensity. The apparatus was a normal aktograph with the rear half covered in clear material and the remainder in dark material. The activity and position of 30 individual slugs were recorded overnight when the apparatus was set up near a laboratory window.

Results

The results of the activity of 4 slugs between 18:00 hrs. and 10:00 hrs. on the following day are given in Fig. 6. The line across each trace represents the boundary of the light and dark sides. It is clear that each slug moved into the light area at dusk, and confined its activities to this side until dawn when it returned to the shaded half where it was more or less inactive. *Arion ater* are therefore very sensitive to small changes in light intensity at night, and this possibly enables them to find

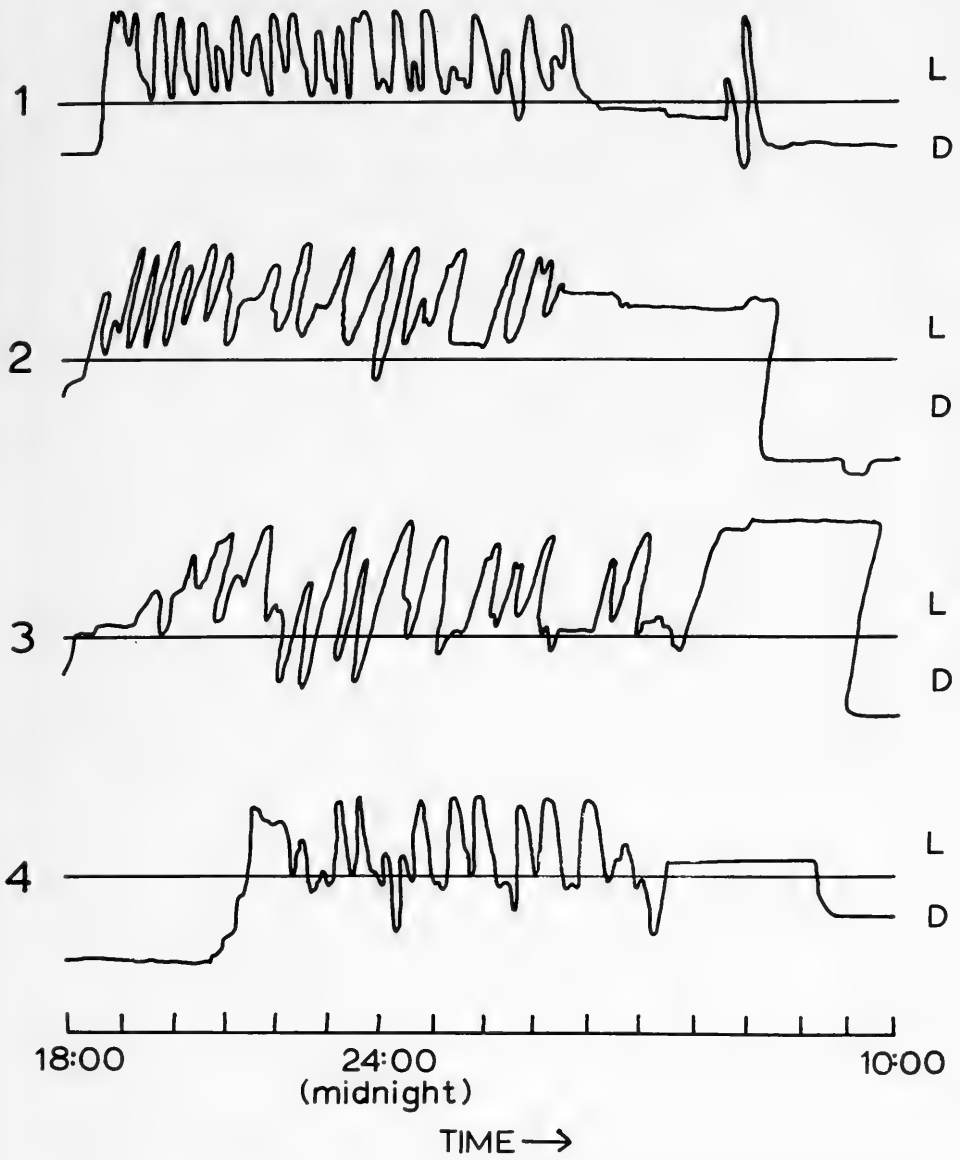

FIG. 6. The activity of *Arion ater* in a light-choice chamber at night. The light (L) and dark (D) sides of the chamber are indicated.

cover at the end of their active phase. During the active phase they showed a preference for dim light, rather than complete darkness.

3. The effect of changes in light intensity on the timing of locomotor activity

Method

If the decrease in light intensity at sunset to any extent controls the timing of the onset of activity, it should be possible to advance this event by subjecting slugs to early artificial darkness. To test this, colonies of 14 slugs were set up in glass-covered experimental chambers out of doors. Some colonies were subjected to early artificial darkness by covering with black plastic sheets before sunset, and an equal number of control colonies remained covered with glass. The number of slugs visible in each chamber was noted at the time of covering and a few hours later. The temperatures of the covered and control chambers did not differ significantly.

Results

The results of 3 of the trials are recorded in Table 1. For each trial the number of slugs visible in both the experimental and control colonies are recorded at the time of covering the experimental colonies, and again within a few hours. These numbers have been summed to give the totals in each colony at both times of counting. In all cases the numbers visible before covering were approximately the same, and at the second count there were always more visible in the covered colonies. Thus the early darkness imposed on the experimental colonies initiated early activity, and so the onset of activity in the field may be controlled by the fall in light intensity at sunset.

IV. THE SYNCRONISATION OF LOCOMOTOR ACTIVITY WITH 24 HOUR CYCLES OF ARTIFICIAL LIGHT

Method

The effect of light on the timing of locomotor activity was studied further by subjecting 6 slugs to 24 hour artificial light cycles. The slugs had been maintained in constant darkness at 10°C for several weeks during which time their endogenous activity patterns were either lost or weakened. Activity was recorded in the aktograph apparatus which was covered with half dark and half clear material providing an area of shade in order to simulate natural conditions as far as possible. The procedure was to record the activity of the slugs in constant conditions for 2 days, and then to subject them to 24 hr. cycles of artificial light. A tungsten lamp was switched on at 09:00 hrs. on the second day, and at 06:00 hrs. on subsequent days, and switched off at 21:00 hrs. each evening.

Results

The results of 1 trial are recorded in Fig. 5; the dark phases of the synchronising cycle are shaded. In this case, as in all the trials, the locomotor activity was synchronised with the light cycles within 4 days, so that the slugs were active mainly at night, and the onset of activity followed the start of the dark phase. It was therefore concluded that light was an important factor in the control of the timing of the activity of *Arion ater*, and it is likely that the timing of field activity is governed to some extent by the natural light cycles.

DISCUSSION

Previous work (Dainton, 1954) has

TABLE 1. The effect of artificial early darkness on the timing of the onset of activity of *Arion ater*[2]

Trial 1

	Day	1	2	3	4	5	TOTAL
EXPERIMENTAL	15:00 hrs.	0	0	0	0	0	0
	17:00 hrs.	1	1	1	2	5	10
CONTROL	15:00 hrs.	0	0	0	0	0	0
	17:00 hrs.	0	0	0	0	0	0

(Container covered at 15:00 hrs., and counted at 15:00 hrs. and 17:00 hrs.)

Trial 2

	Day	1	2	3	4	TOTAL
EXPERIMENTAL	17:00 hrs.	0	2	0	0	2
	21:00 hrs.	7	8	8	6	29
CONTROL	17:00 hrs.	0	2	1	0	3
	21:00 hrs.	7	4	2	2	15

(Container covered at 17:00 hrs., and counted at 17:00 hrs. and 21:00 hrs.)

Trial 3

	Day	1	2	3	4	TOTAL
EXPERIMENTAL	19:00 hrs.	0	0	0	0	0
	20.30 hr.	6	10	3	7	26
CONTROL	19:00 hrs.	0	0	0	0	0
	20:30 hrs.	0	1	0	0	1

(Container covered at 19:00 hrs., and counted at 19:00 hrs. and 20:30 hrs.)

[2]The numbers of slugs visible at the times of counting as indicated in the table are given for each day of the experiment, together with totals for the whole experiment.

shown that the timing of the activity of the slug *Agriolimax reticulatus*, and perhaps by implication other species, was controlled by the temperature fluctuations of the environment, and that light and humidity were of little importance. However, more recent work by Newell (1968) on the same species showed a closer connection between activity and light intensity rather than with temperature. In contrast with Dainton's conclusions on the control of activity in *Agriolimax reticulatus*, it was found in the present work that locomotor activity rapidly sychronised with 24 hr. artificial light cycles, and that similar temperature cycles failed to produce any effect. This indicates that for *Arion ater* the environmental light cycles are probably the most important of the exogenous controlling factors, although factors, such as changes in the earth's magnetic field, which were not tested, may also have a similar effect.

The results of the humidity-choice experiments, although not unexpected, give clear evidence of the preference shown by *Arion ater* for humid environments. The humidity differences in the choice-chambers were large and in excess of any differences slugs would encounter in the field, but it is thought likely that the same reactions are shown in the field. It is therefore possible that slug movement is limited by the humidity of the air bordering the substratum, but it should be pointed out that this is frequently saturated at night, so that low humidity alone would only infrequently limit activity. Wind undoubtedly has some effect on this as it disturbs the air near the ground and increases evaporation from the skin. Consequently it is expected that wind in conjunction with low humidity reduces slug activity.

ACKNOWLEDGEMENTS

I am grateful to Professor James Brough for providing facilities in the Department of Zoology, University College, Cardiff, where the work was carried out. I would like to thank Dr. W. A. L. Evans for his help and advice throughout the investigation and in the preparation of the script. The work was carried out under a Science Research Council Research Studentship.

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RÉSUMÉ

ETUDES SUR L'ACTIVITÉ LOCOMOTRICE DE LA LIMACE *ARION ATER* (LINNAEUS)
I RÉACTIONS VIS-À-VIS DE L'HUMIDITÉ, DE LA TEMPÉRATURE ET DE LA LUMIÈRE

R. D. Lewis

Les réactions de la limace *Arion ater* (L.) vis-à-vis de l'humidité, de la température et de la lumière sont étudiées. Les méthodes employées font appel à un simple actographe pour enregistrer l'activité locomotrice. Celles-ci mettent en évidence que l'adulte d'*Arion ater* est capable de distinguer entre forte et faible humidité, et montre en général une préférence pour une forte humidité.

En laboratoire, les changements de température n'ont pas d'effets sur la période d'activité locomotrice, aussi conclut-on que le champ d'activité n'est pas contrôlé par les cycles naturels de température. *Arion ater* synchronise très vite son activité sur des cycles de lumière artificielle de 24 heures, aussi suggère-t'on que la lumière naturelle est le facteur d'environnement le plus important dans le contrôle du champ d'activité.

RESUMEN

ESTUDIOS DE LA ACTIVIDAD LOCOMOTORA EN LA BABOSA *ARION ATER* (LINNAEUS)
I. HUMEDAD, TEMPERATURA Y REACCION A LA LUZ

R. D. Lewis

Los métodos usados emplearon un aparato aktografico simple para registrar actividad locomotora, y demostraron que el adulto de *Arion ater* es capaz de distinguir entre grados de humedad altos y bajos, con general preferencia por alta humedad.

Cambios de temperatura en el laboratorio no tuvieron efecto en la regulación del tiempo de la actividad locomotora, por lo que se estima que, en ambiente natural, esa actividad no está controlada por ciclos térmicos.

Arion ater sincroniza su actividad con ciclos de 24 horas de luz artificial y esto sugiere que la luz natural es el factor ambiental más importante en el control de la actividad.

АБСТРАКТ

ИЗУЧЕНИЕ ДВИГАТЕЛЬНОЙ АКТИВНОСТИ СЛИЗНЯ ***ARION ATER***
I. РЕАКЦИЯ НА ВЛАЖНОСТЬ, ТЕМПЕРАТУРУ И СВЕТ

Р.Д.ЛЬЮИС

Изучались реакции слизня ***Arion ater*** **(Linnaeus)** на влажность, температуру и свет. Для регистрации двигательной активности использовался простой актограф. Показано, что взрослые ***Arion ater*** способны различать высокую и низкую влажность и обычно предпочитают высокую.

В лабораторных условиях изменения температурыне оказывает влияния на синхронизацию двигательной активности, откуда делается вывод, что активность в природе не контролируется естественными температурными циклами. ***Arion ater*** легко синхронизирует свою активность с 24-х часовьм циклом естественной освещенности и поэтому высказывается предположение, что естественный свет является наиболее важным фактором среды, контролирующим активность в природе.

MALACOLOGIA, 1969, 7(2-3): 307-312

STUDIES ON THE LOCOMOTOR ACTIVITY OF THE SLUG *ARION ATER* (LINNAEUS)

II. LOCOMOTOR ACTIVITY RHYTHMS

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ABSTRACT

The extent to which the timing of the locomotor activity of the slug *Arion ater* is controlled endogenously has been investigated using a simple aktograph apparatus to record activity. In constant humidity, constant temperature and darkness, *A. ater* exhibits rhythmic locomotor activity in which the period of the rhythm is less than 24 hrs. and decreases as time goes on. This rhythm does not follow abnormal light cycles, and this is taken as evidence that it is truly endogenous.

It is now generally accepted that numerous examples of rhythmic activity exhibited by animals and plants may be derived from true internal timing mechanisms. These function in an organism to synchronise numerous physiological processes between themselves and with the environment (Harker, 1964). Although it is frequently easy to record rhythmic activity in what are thought to be constant conditions, there is always the possibility that the recorded rhythm is the result of synchronisation with some unknown exogenous factor, and it is often necessary to carry out further complex experiments to prove conclusively that the recorded rhythm is truly endogenous.

It has been shown (Lewis, 1969) that the timing of the locomotor activity of *Arion ater* is controlled to some extent by the environmental light cycles, although it is possible that overall control is through an interaction with an endogenous timing mechanism. Experiments were therefore devised in which the locomotor activity of *A. ater* was recorded in constant conditions over a number of days.

I. ACTIVITY IN CONSTANT CONDITIONS

Method

The locomotor activity of 13 freshly collected *Arion ater* was recorded using a simple aktograph apparatus (Lewis, 1969) in complete darkness at 10° C for 2 weeks or more.

Results

The aktograph traces of the activity of 1 slug over 21 days are presented in Fig. 1, and in Fig. 2 the times of onset of activity of another slug are plotted for each day for the duration of the recording. The results clearly demonstrate that under the conditions of the experiment the slugs exhibited rhythmic locomotor activity in which the time of the onset of activity was earlier each day. Not all the slugs tested showed clear rhythmicity at all times, but most were rhythmic for several days, and some continued for much longer. The curve in Fig. 2 shows that the time of onset of activity advanced each day so that the period of the rhythm was in-

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itially 23 hrs. but decreased to about 20 hrs. before it broke down.

This rhythmic activity was recorded in constant temperature, in a saturated atmosphere and in complete darkness, but it was realised that daily changes in the earth's magnetic field, were not excluded. From the results, therefore, one cannot be sure that the recorded rhythm was truly endogenous as there may have been external synchronisation. It was consequently necessary to carry out further experiments to provide more conclusive evidence on this point. These were based on the finding (Lewis, 1969) that it was possible to synchronise slug activity with 24 hr. cycles of artificial light. It was argued that if there were no endogenous control of locomotor activity then it was reasonable to expect synchronisation with short period light cycles. If, on the other hand, there was some endogenous control of activity then it would be expected that locomotor activity would not follow a light cycle with periodicity differing greatly from 24 hrs. Two further experiments were carried out with *Arion ater;* the first was to show the effect of a short period artificial light cycle on activity, and the second was to record the reaction to a single large phase change in a 24 hr. light cycle.

II. SYNCHRONISATION WITH 18.5 HOUR LIGHT CYCLES

Method

In this experiment to test the possibility of synchronising slug activity with short period light cycles in constant temperature, the locomotor activity of 5 freshly collected field slugs was recorded at 13° C in an aktograph with a cover of half dark and half clear material, first for 2 days when subjected to 24 hr. artificial light cycles, and then under 18.5 hr. light cycles with equal light and dark phases. The phasing of the 24 hr. cycles closely followed natural conditions so that the activity was synchronised with them at once.

Results

The results, one of which is recorded in Fig. 3, clearly demonstrated that having synchronised with the 24 hr. cycles, the activity failed to follow the shorter period cycle but became more or less random as time went on. This suggested that the rhythm recorded in constant conditions was truly endogenous.

III. THE RESPONSE TO A LARGE PHASE CHANGE

Method

As a further test of the endogenous control of locomotor activity in *Arion ater*, their response to a 6 hour phase change in a synchronising cycle was recorded in constant temperature. First the activity of several freshly collected slugs was synchronised with 24 hr. artificial light cycles in the aktograph as in the previous experiment. After 2 days the time of the dark phase of the synchronising cycle was brought forward by 6 hours on 1 occasion, and the same frequency and phasing were continued to the end of the experiment.

Results

The response of 1 slug is given in Fig. 4; on the first 2 days the activity followed the imposed 24 hr. cycle, but as soon as the phasing was altered it tended to become random, although the continuation of the original rhythm can be seen for some time. In some examples even after 12 days under the new conditions there was no evidence of resynchronisation. It was concluded that this response gives further evidence for the endogenous control of locomotor activity in *Arion ater*.

DISCUSSION

Before discussing the significance of the endogenous rhythm in the field be-

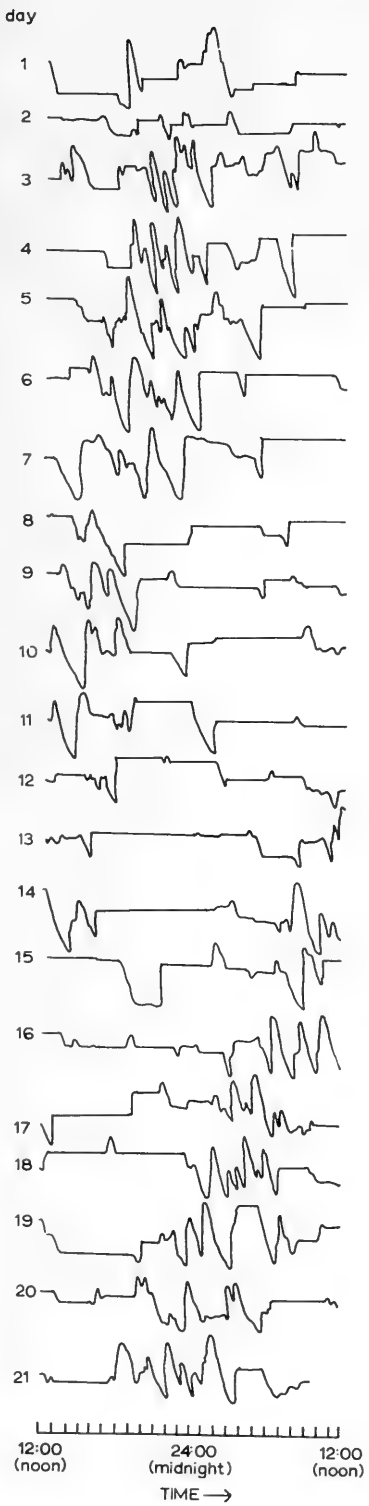

FIG. 1. The locomotor activity of *Arion ater* in constant conditions for 21 days.

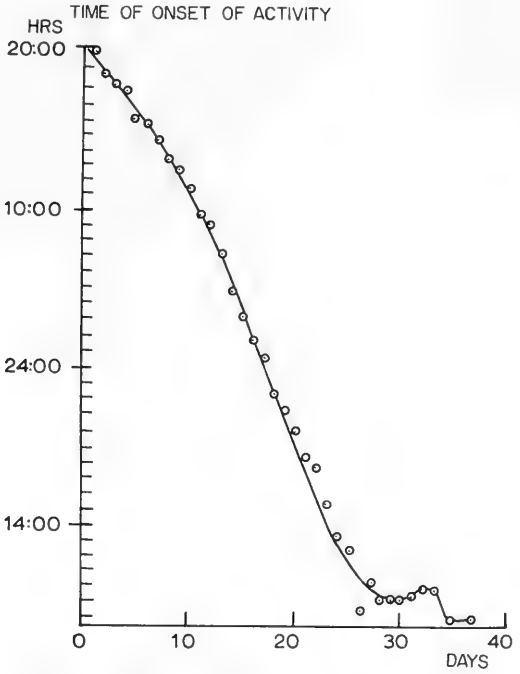

FIG. 2. The times of onset of locomotor activity of one *Arion ater* in constant conditions for many days.

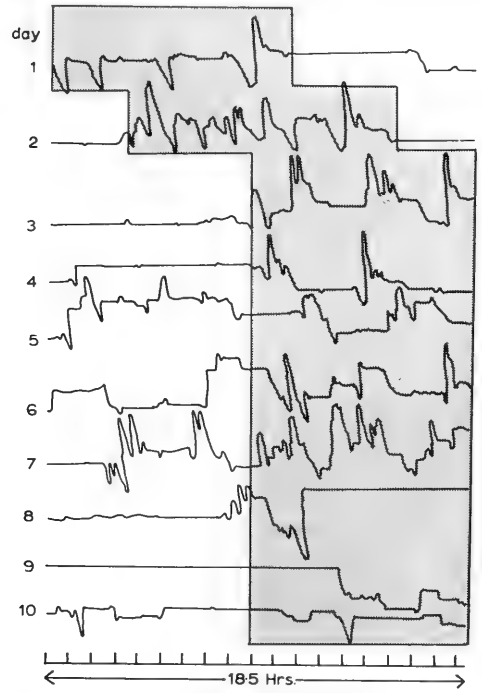

FIG. 3. The response of *Arion ater* to 18.5 hr. cycles of artificial light. For the first 2 days locomotor activity was synchronised with 24 hr. light cycles. The dark phases of the light cycles are shaded.

haviour of *Arion ater*, it is worthwhile to consider the evidence for the endogenous, rather than exogenous, control of the rhythmic activity. The conditions under which the rhythm was originally recorded only prevented variations in the most obvious sychronisers - temperature, light and humidity - and did not exclude the effects of changes in barometric pressure, cosmic rays and the earth's magnetic field. The recorded rhythm initially had a period of 23 hrs., and this later reduced to about 20 hrs., but if in these experimental conditions the activity was synchronised by one of the less obvious factors it would seem reasonable to expect the rhythm to continue with the same frequency as field activity, i.e., about 24 hrs. Also it has been shown (Lewis 1969) that light was the main environmental factor in the control of the timing of field activity, and moreover it was possible to synchronise activity with 24 hr. cycles of artificial light. Thus, if control was entirely exogenous it would be expected that activity would follow abnormal light cycles. Since *A. ater* did not react in this way, it was concluded that the rhythm was truly endogenous.

It is not unusual for the period of a biological rhythm, such as this, to differ from 24 hrs. by up to 3 hours (Harker, 1964), and it is generally not possible to synchronise these rhythms with cycles outside this range. Not surprisingly light is an effective synchroniser for many rhythms, although both temperature (for rats, salamanders and cockroaches) and feeding times (for bats) also act as synchronisers (Harker, 1964). It is unusual for the period of biological rhythms to vary consistently, as it did with *Arion ater*, and the reason for this is not known, but it may have been the result of physiological changes in the slugs.

The significance of the rhythm in field behaviour becomes clear when it is realised that *Arion ater* remain under cover during the daytime, and are not subjected to the changes in the environment as a whole. They are therefore unable to estimate the time of day from their surroundings. It should also be remembered that as slugs are liable to desiccation if they expose themselves in unfavourable conditions, they cannot appear from cover unless conditions are suitable for activity. Thus it is considered that they remain inactive under cover until rather less than 24 hrs. after the start of the previous active phase. As a result of their internal rhythm they then move, and are able to determine if conditions are suitable for activity. Newell's (1966) time-lapse films of the behaviour of *Agriolimax reticulatus* at sunset show that these slugs frequently extend their tentacles at the exit of their cover, which in this species is a hole in the ground, and then withdraw if conditions are unsuitable. The light intensity is probably the most important factor in determining the precise time of the onset of activity in *Arion ater*, but other factors, such as wind, humidity and precipitation, may influence the length of the active phase. The return to cover is probably controlled by a homing behaviour pattern and the ability to distinguish between small differences in light and shade at night. On the rare occasions when activity is continued throughout the day, other physiological factors, in conjunction with dull and damp weather, may over-ride the usual reactions.

ACKNOWLEDGEMENTS

I am grateful to Professor James Brough for providing facilities in the Department of Zoology, University College, Cardiff, where the work was carried out. I would like to thank Dr. W. A. L. Evans for his help and advice throughout the investigation and in the preparation of the script. The work was carried out under a Science Research Council Research Studentship.

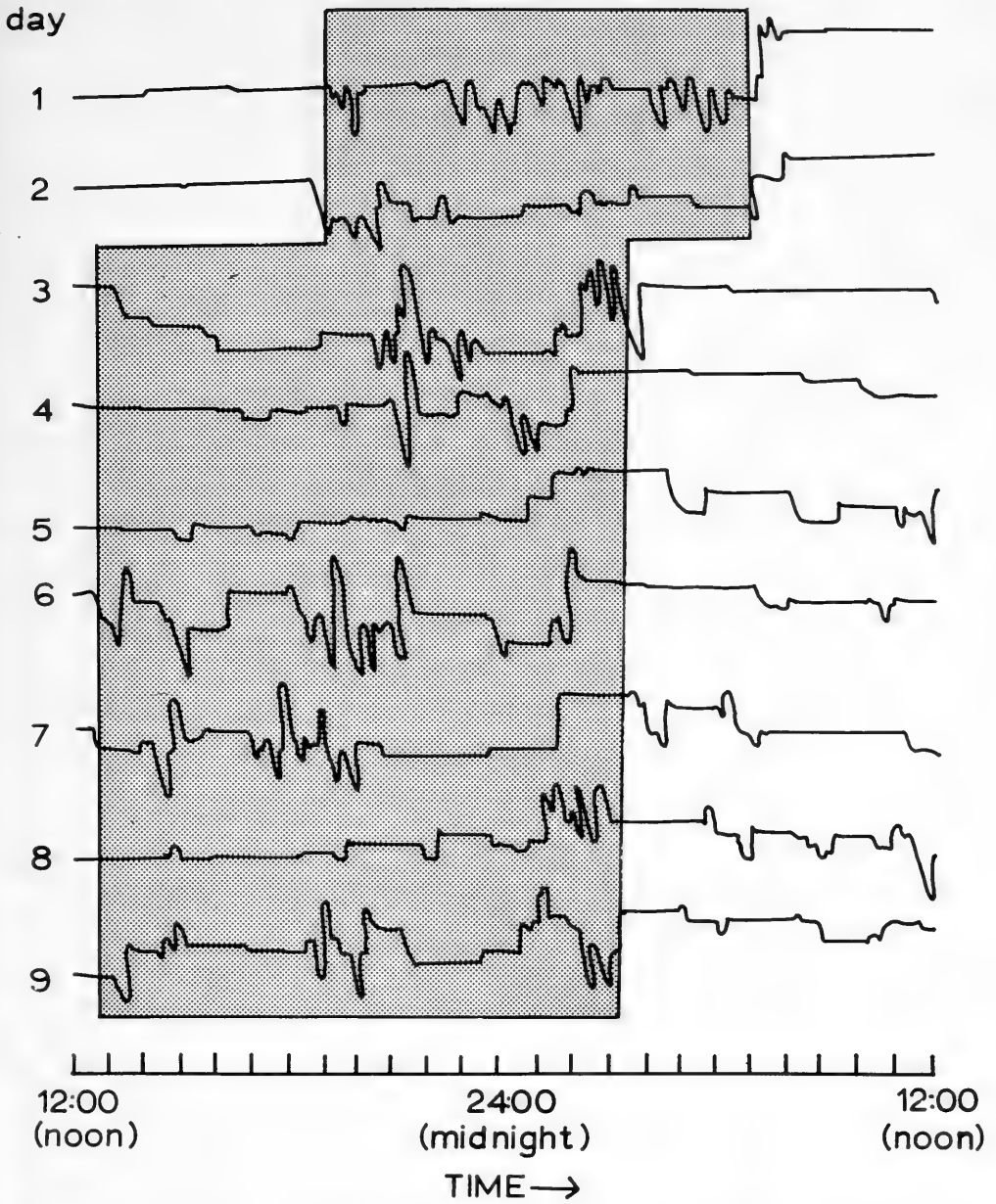

FIG. 4. The effect of a large phase change in a synchronising light cycle on the timing of the locomotor activity of *Arion ater*. The dark phases of the light cycles are shaded.

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RÉSUMÉ

ETUDES SUR L'ACTIVITÉ LOCOMOTRICE DE LA LIMACE *ARION ATER* (LINNAEUS)
II RYTHMES D'ACTIVITÉ LOCOMOTRICE

R. D. Lewis

L'hypothèse selon laquelle le temps d'activité locomotrice d'*Arion ater* est contrôle de façon endogène, a été examinée à l'aide d'un simple actographe, de façon à enregistrer l'activité. Lorsque l'humidité, la température et l'obscurité sont constantes, *Arion ater* montre une activité locomotrice rythmique dans laquelle la période des rythmes est de moins de 24 h et décroit à mesure que le temps passe. Ce rythme ne suit pas les cycles anormaux de luminosité, ce que l'on considère comme l'évidence même qu'il est bien endogène.

RESUMEN

ESTUDIOS DE LA ACTIVIDAD LOCOMOTORA EN LA BABOSA *ARION ATER* (LINNAEUS)
II. RITMO DE ACTIVIDAD LOCOMOTORA

R. D. Lewis

El alcance del control endógeno, en la regulación del tiempo de la actividad locomotora en *Arion ater*, fué investigado usando para su registro un aparato aktografico simple. En humedad, temperatura y obscuridad constantes, *A. ater* tiene un movimiento rítmico que es menor de 24 horas, y va decreciendo en tales condiciones. Este ritmo no sugiere ciclos luminosos anormales y se toma como evidencia de que es verdaderamente endógeno.

АБСТРАКТ

ИЗУЧЕНИЕ ДВИГАТЕЛЬНОЙ АКТИВНОСТИ СЛИЗНЯ *ARION ATER*
II. РИТМЫ ДВИГАТЕЛЬНОЙ АКТИВНОСТИ

Р.Д.ЛЬЮИС

С помощью простого актографа для регистрации активности было установлено до какой степени синхронизация двигательной активности слизня *Arion ater* контролируется эндогенно. При постоянной влажности, постоянной температуре и в темноте *A. ater* обнаруживает ритмичную двигательную активность, период ритма которой менее 24-х часов и со временем укорачивается. Аномальные световые циклы не влияют на ритм, что является доказательством того, что он чисто эндогенный.

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THE ZOOGEOGRAPHY AND ECOLOGY OF ARIONID AND LIMACID SLUGS INTRODUCED INTO NORTHEASTERN NORTH AMERICA

Lyle F. Chichester[1] and Lowell L. Getz[2]

ABSTRACT

A study was made of the geographical and ecological distribution of 12 species of imported arionid and limacid slugs in northeastern North America. A total of 770 sites were visited in 8 states and 5 provinces, of these, 510 sites yielded at least 1 imported species. Three species (*Deroceras reticulatum, Arion fasciatus* and *A. subfuscus*) were found to be very abundant. The status of the 3 morphological components of the *A. fasciatus* complex is discussed; these components are treated as separate species throughout the study. New locality records were obtained for *A. intermedius, A. hortensis, A. subfuscus* and *D. caruanae*.

The 3 most widely distributed species show different ecological preferences. *Arion subfuscus* is primarily a woodland slug. *D. reticulatum* is essentially a field slug. *Arion fasciatus* is most abundant in ecotonal situations. Of the 3 species, *A. subfuscus* is most widely distributed in natural areas. It occurs in great abundance in the Catskill and Adirondack Forest Preserves of New York State, forested regions of northern New England, Nova Scotia and parts of Quebec.

An attempt was made to determine the probable mechanisms of importation and dispersal which have led to the present day distribution. The basic pattern is that most, if not all, species have been introduced on more than one occasion. The most probable means of introduction was with imported plant materials. Although it was not possible to eliminate ballast dumping as a source of European slugs in certain coastal areas, it would appear that that was not the major method of importation. Apparently the most common portals of entry were large, long established urban areas. From these primary sites slugs were transported to secondary sites, probably in plant materials, from which they dispersed by both active and passive means. Several species show considerable ability to actively disperse in suitable habitats. This ability has been discounted by previous authors.

CONTENTS

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INTRODUCTION

The first report of an imported European slug in North America was by Thomas Say prior to 1822 (Binney, 1842). Since then, the number of imported arionid and limacid species has increased to 15. There are many published locality records for these species (cf. Chichester & Getz, 1968a). Most of the records are from greenhouses, gardens, urban areas and other cultivated areas. It is generally assumed that slugs have been introduced on plant materials imported from European gardens, nurseries and greenhouses. Lindroth (1957) has suggested, however, the possibility of introduction by means of ballast dumping to explain the presence of European slugs and snails on small, deserted islands off the coast of Newfoundland's Avalon Peninsula. It is also assumed that imported slugs disperse chiefly by passive means, such as transport on nursery stock or greenhouse plants. No one has examined the distribution of imported slugs on a regional basis to determine to what extent they may be established in other than cultivated areas. Nor has anyone attempted to test the assumptions concerning the method of importation or the mechanisms of dispersion.

Preliminary collecting (Getz, 1962a; Getz & Wakefield, 1963) indicated certain European slugs were relatively abundant in some regions of the northeast and that at least one species had dispersed into natural areas. A more extensive survey of the distribution of imported European slugs was therefore undertaken.

The principal goals of the present study were to determine: (1) the present distribution of all imported arionid and limacid slugs in northeastern North America, (2) the probable method(s) of introduction for each species, (3) the extent to which these species have spread to natural (uncultivated or wild) areas under their own active dispersal powers.

For the purposes of this study, northeastern North America includes: New England, New York, the extreme northeastern corner of Pennsylvania, Newfoundland (Labrador), Nova Scotia, New Brunswick (including Prince Edward Island), Quebec and the eastern half of Ontario.

Only imported slugs in the families Arionidae and Limacidae are considered in detail; distributional data on native Philomycidae (*Philomycus carolinianus* and *Pallifera dorsalis*) and Limacidae (*Deroceras laeve*) are presented as a basis for comparison.

DISTRIBUTION OF EUROPEAN SLUGS

I. World distribution

Several species of European slugs have achieved almost worldwide distribution in the space of a few hundred years through accidental importations promoted by intercontinental and international commerce. All of the following slugs have been introduced into Australia, South Africa and South and North America: *Limax maximus*, *L. flavus*, *Lehmannia valentiana* and *Deroceras reticulatum*. *Milax gagates* probably also belongs in this list, though some records for this species may actually refer to *M.* cf. *insularis* (Quick, 1960). All of these slugs are characteristically associated with cultivated areas such as gardens, fields, greenhouses, cellars, stone walls and dumps.

A second group, not quite so widely distributed as the first, consists of slugs commonly found in both cultivated and

natural woodland or ecotonal habitats. Of these slugs, *Arion hortensis* is probably most widely distributed, having been introduced into Australia, New Zealand, Tasmania, South Africa and North America. It is also the most "domesticated" slug in the group. This group also includes *A. subfuscus*, *A. ater*, *A. intermedius*, the *A. fasciatus* complex, and *Lehmannia marginata*.

Deroceras caruanae has been omitted from both lists because its distribution is poorly known. On the basis of what little is known of its ecology (i.e., it inhabits cultivated areas), one would expect that it belongs to the first group.

There is a third group of European slugs characterized by complete failure, thus far, to achieve intercontinental importation. In general, slugs in this group are either not widely distributed (e.g., *Geomalacus maculosus*) or else are restricted to woodlands and other wild areas (e.g., *Limax cinereoniger*). It would seem that for a species to have a world-wide distribution by means of accidental importation it should show a tendency to select cultivated habitats and should be widely distributed in its native region.

II. North American distribution

Since the first report of an introduced slug at least 15 species of European slugs (not including Testacellidae) have been found in North America (the total includes *Limax nyctelius* Bourguignat which has been reported in North America only once, from a Washington, D.C. greenhouse; Quick, 1960). Table 1 summarizes the previously known history of introductions into northeastern North America of 12 species (2 others are reported for the first time in this paper). Additional comments follow below.

Arion subfuscus (Draparnaud). Later records for this species suggested that it was uncommon in much of the Northeast. Brooks & Brooks (1940) give additional Newfoundland records, while MacMillan (1954), Moore (1962) and Dimelow (1962b) discuss the distribution of *A. subfuscus* in Nova Scotia. Karlin & Naegele (1960) furnish 6 greenhouse records from central and western New York.

Arion fasciatus complex. The complex has subsequently been reported from most of Canada and much of the United States. Unfortunately all records in North America fail to indicate which species was involved. The complex consists of 3 species: *Arion fasciatus* (Nilsson), *A. circumscriptus* Johnston and *A. silvaticus* Lohmander. Lohmander (1937) was the first to understand that what had previously been treated as a single species under the name *Arion fasciatus* (or *A. circumscriptus*) was actually a complex of 3 species. He pointed out that these species differed both morphologically and ecologically. Walden (personal communication) has conducted extensive morphometrical studies which support the idea of a 3 species complex. Chichester (1967) did an electrophoretic study of the albumen gland proteins of all 3 species in the complex. These results also support Lohmander's original ideas.

Arion hortensis Ferussac. In addition to records from the Northeast, *A. hortensis* was reported from California in 1940 (Pilsbry, 1948). Johnson's (1915) Connecticut record may refer to *Arion fasciatus*, as did Morse's (1864) Maine record. Although reported from several localities, this species is relatively uncommon in North America.

Arion intermedius (Normand). The only other records of this species are from a number of localities in California (Pilsbry, 1948).

Arion ater (Linnaeus). First reported in North America by Walker from Detroit, Michigan in 1912; other records outside the Northeast include Oregon (Pilsbry, 1948), California and Washington (Smith, 1962), and British Columbia (LaRocque, 1953). O'Neil (1964) gives additional records of *A. ater* from Quebec.

TABLE 1. First reports of European slugs from each state and province of northeastern North America. [P], first cited by Pilsbry, 1948; ?, doubtful record; *, first record is from the present study; o, species not yet reported from state or province

Species	State or Province		
	Maine	New Hampshire	Vermont
Arion subfuscus	*	Getz, 1962a; several sites	*
A. fasciatus Complex	Clapp, 1905[P]; Kennebunkport	Getz & Wakefield, 1963; Campton	*
A. hortensis	o	o	o
A. intermedius	*	*	o
A. ater	Clench, 1928[P]; Basin Falls	o	o
Limax maximus	Lermond, 1909; Bar Harbor	o	o
L. flavus	Morse, 1864; Portland	o	o
Lehmannia valentiana	*	o	o
L. marginata	o	o	o
Deroceras reticulatum	Morse, 1864; Portland	Dall, 1916?; Mt. Monadnock	*
D. caruanae	o	o	o
Milax gagates	o	o	o
	Massachusetts	Connecticut	Rhode Island
Arion subfuscus	A. Binney, 1842[P]; Boston	*	o
A. fasciatus Complex	Moore, 1902[P]; Woods Hole	Getz & Wakefield, 1963; Storrs	o
A. hortensis	A. Binney, 1842; Boston	Johnson, 1915?; New Haven	o
A. intermedius	*	*	*
A. ater	o	Getz & Wakefield, 1963; Storrs	o
Limax maximus	Pilsbry, 1882[P]; Cambridge	*	Powel, 1868[P]; Newport
L. flavus	W. Binney, 1851[P]; Boston Area	Johnson, 1915; New Haven	o
Lehmannia valentiana	Walden, 1961; Belmont	*	o

Table 1 (cont.)

Species	State or Province		
	Massachusetts	Connecticut	Rhode Island
L. marginata	o	o	o
Deroceras reticulatum	A. Binney 1842; Boston	Johnson, 1915; 2 sites	o
D. caruanae	o	o	o
Milax gagates	o	o	o
	New York	Pennsylvania	Nova Scotia
Arion subfuscus	Robertson, 1941[p]; East Aurora	Pilsbry, 1940[p]; Haverford	Ord & Watts, 1949; 36 sites
A. fasciatus Complex	Smith & Prime, 1870[p]	Frazen 1947[p]; Del. Water Gap	LaRocque 1937[p]; Morden, Berwick
A. hortensis	Letson, 1905; Poughkeepsie	Clapp 1905[p]; Pittsburgh	Ord & Watts 1949; 10 sites
A. intermedius	Tesky 1951; Buffalo	o	o
A. ater	o	o	o
Limax maximus	Pilsbry 1870[p]; Long Island	Tryon, 1867[p]; Philadelphia	o
L. flavus	Dekay 1843	Say, prior to 1822[p]; Philadelphia	o
Lehmannia valentiana	Karlin & Naegele, 1960; 68 sites	Walden, 1961; Hartsville	*
L. marginata	o	o	o
Deroceras reticulatum	A. Binney, 1842; New York	A. Binney, 1842; Philadelphia	Campbell, 1906; Pictou Area
D. caruanae	o	o	o
Milax gagates	Karlin & Naegele, 1960; 12 sites	Clapp, 1906[p]; Pittsburgh	Fox, 1962; Amherst
	New Brunswick	Quebec	Ontario
Arion subfuscus	Dimelow, 1962b; Sackville	Ball, 1939[p]; Gaspé Area	*
A. fasciatus Complex	LaRocque, 1961; not noted	LaRocque, 1936[p]; 2 sites	Robertson, 1913[p]; Toronto

Table 1 (cont.)

Species	State or Province		
	New Brunswick	Quebec	Ontario
A. hortensis	o	Pennisi, before 1948[p]; Gaspé Area	Oughton, 1948; Toronti, Wiarton
A. intermedius	o	*	o
A. ater	o	Ball, 1938[p]; Gaspé Area	o
Limax maximus	o	o	Latchford, 1904; Ottawa
L. flavus	o	o	o
Lehmannia valentiana	*	*	o
L. marginata	o	o	o
Deroceras reticulatum	Dimelow, 1962b; Sackville	Latchford, 1885a; Quebec	Latchford, 1885b; Ottawa
D. caruanae	o	*	o
Milax gagates	o	o	o
	Newfoundland	Labrador	Prince Edward Island
Arion subfuscus	Brooks, 1936; 2 sites	o	o
A. fasciatus Complex	Brooks & Brooks, 1940; 8 sites	o	Long, 1912[p]; Charlottetown
A. hortensis	Long, 1926[p]; 2 sites	o	o
A. intermedius	o	o	o
A. ater	Long, 1924[p]; Bay Bulls	o	o
Limax maximus	Brooks & Brooks, 1940; 2 sites	o	o
L. flavus	o	o	o
Lehmannia valentiana	o	o	o
L. marginata	Brooks & Brooks, 1940; 7 eastern sites	o	o
Deroceras reticulatum	Long, 1927[p]; several sites	Packard, 1867; 2 sites	Long; date and locality not noted[p]
D. caruanae	o	o	o
Milax gagates	o	o	o

Limax maximus Linnaeus. This species has now been reported from many states throughout North America; early records include Ohio, 1882; Texas, 1886; and California, 1900 (Pilsbry, 1948).

Limax flavus Linnaeus. Also reported from Virginia and Maryland prior to 1851; it has subsequently been reported from several other states (Pilsbry, 1948).

Deroceras reticulatum (Müller). This species occurs throughout most of the United States and Canada; it is the most abundant imported species in cultivated areas of the Northeast.

Deroceras caruanae (Pollonera). Only previous record is from California (Pilsbry, 1948).

Lehmannia valentiana (Ferussac). First reported (as *Limax marginatus*) from a Colorado greenhouse in 1917 by Cockerell and was found widely distributed in natural habitats in California in 1930 (Pilsbry, 1948). It has since been reported from greenhouses in many other states. An unpublished Master's thesis (Sivik, 1953), which was supposed to be a study of the biology and control of *Deroceras reticulatum*, apparently refers to *L. valentiana*. Photographs in the thesis were clearly not *D. reticulatum*, but were typical *L. valentiana;* his greenhouse records of *D. reticulatum* in Massachusetts probably refer to *L. valentiana*.

Lehmannia marginata (Müller). The only confirmed report of this species (as *Limax arborum*) in North America is by Brooks & Brooks (1940) in Newfoundland. All other reports of *L. marginata* are assumed to belong to *L. valentiana* (cf. Walden, 1961).

Milax gagates (Draparnaud). First reported in California in 1872. It has subsequently been reported in other West Coast states and in Virginia, as well as in the northeast.

III. Early History of northeastern North American introductions

Amos Binney (1842) provided the first report of European slugs in the region. He apparently did sufficient field work to note that the slugs he encountered displayed 2 distinctly different distribution patterns. One group (*Deroceras laeve*, *Pallifera dorsalis* and *Philomycus carolinianus*) was widely distributed throughout New York and New England. He considered these slugs native. A second group was found only around the maritime cities, especially Boston. It was apparently this limited distribution pattern, rather than an aforehand acquaintance with European slugs, that led him to conclude they must be imports. He found *Arion* (probably 2 species[3]) "in small numbers in company with *Limax agrestis* under stones at roadsides." Of *Deroceras reticulatum* (*Limax agrestis*) he said it "has not yet penetrated far into the interior of the country. Common in the neighborhood of Boston under stones at roadsides and about stables and farm yards and, in other moist situations, under decaying pieces of wood. It is also found in "cellars and gardens" where he noted they did not cause much damage. He also noted that *D. reticulatum* was quite variable with respect to color. DeKay

[3]Binney reported the slug as *A. hortensis*. He described the slug as follows: "color of upper surface whitish or light ashy, sometimes with a slight tinge of brown; an obscure, ill-defined brownish line extending along the lower margin of mantle and of the body on both sides." This description fits *A. subfuscus* far better than *A. hortensis*. In another part of his paper Binney says that his slug agrees with Férussac's description of *A. hortensis* as "griseus, unicolor, fasciis nigris." This obviously contradicts his earlier statement. However, a possible explanation is provided by his son, W. Binney (1885), who indicated that two forms of *A. hortensis* (=*A. fuscus*) were present in Boston. One was large (over two inches); the other was small and had a tinge of yellow on the foot sole. It is very likely that Amos Binney found both *Arion subfuscus* and *A. hortensis* in Boston prior to 1842.

(1843) reported *L. flavus* and *D. reticulatum* (as *L. agrestis*) as being found in New York. Morse (1864) reported that *D. reticulatum* (as *L. agrestis*) was "common in fields and by the roadside near villages" in Maine. He noted that *L. flavus* was rarely found in Portland. Morse also reported *A. fuscus* (=*A. hortensis* ?) as a rare inhabitant of gardens in Portland. Since he described only the radular characters, there is reasonable doubt as to which species he had. (Lermond, 1909, listed *Arion fuscus* Morse 1864 as a synonym of *A. circumscriptus* Johnston.) Gould & W. G. Binney (1870) incorporated Tryon's 1867 report of *L. maximus* in Philadelphia and added Rhode Island as the second record. *L. flavus* was also reported from Boston and Cambridge. W. G. Binney (1885) in his manual of American land shells devoted considerable space to both native and imported slugs. However, aside from a few additional locality records, he contributed very little new information.

Johnson (1915) noted that *Limax maximus* was gradually spreading inland from the seaports, though he gives only 8 localities, 5 of which are seaports. He noted that *L. flavus* was confined to seaports and reported *Agriolimax agrestris* (sic) = *Deroceras reticulatum* was widely distributed, although he gives only 9 records, mostly from coastal cities and towns. He reports *A. hortensis*, which he equates with *A. fuscus* of Gould. However, W. G. Binney wrote Gould's description of *A. fuscus;* and the *A. fuscus* of W. G. Binney is the same as *A. hortensis* of A. Binney. Johnson gives 8 localities for Maine, Massachusetts and Connecticut, but again it is doubtful whether all of these records really apply to *A. hortensis* Férussac.

There are also several papers in which the failure to note the presence of slugs may be significant. However, one problem encountered in interpreting the older literature is that many snail workers simply ignored the slugs; so that the absence of slugs from a snail list doesn't necessarily mean that slugs were absent from the region. Both Bailey (1903) in his paper on the land snails of New Brunswick and Jones (1877) in his list of snails from Halifax, Nova Scotia failed to mention even the native slugs. On the other hand Adams (1842), discussing the snails of Vermont, treats the 3 native slug species. His failure to mention European slugs would suggest that they were absent or rare in Vermont in 1842.

MECHANISM OF IMPORTATION AND DISPERSION

On both paleontological and zoogeographic grounds the subfamily Arioninae is considered to be strictly western Palearctic (Pilsbry, 1948; Quick, 1952). This also holds true for the Limacidae, with the exception of the genus *Deroceras* which appears to have a natural holarctic distribution. Only one paper seriously suggests that European slugs in Newfoundland and on some small, uninhabited islands off the Avalon coast may be indigenous (Brooks & Brooks, 1940). Lindroth (1957) suggested, however, that terrestrial mollusks found on offshore islands may have arrived in ballast dumped by British fishing vessels operating from the Avalon coast. This method of introduction might also be responsible for the European slugs found throughout the Northeast.

Most authors who have attempted to account for the introduction of European slugs have concluded, however, that entry has been gained fortuitously in nursery and garden materials. The slug collection of the Canadian National Museum included several specimens of *Arion ater*, *A. subfuscus* and *A. fasciatus* obtained from the Plant Protection Division, which had been taken from imported plant materials.

Several slug species are very widely distributed in the Northeast. This can be accounted for by multiple importations or by rapid dispersal after a single, or a few importations. If the

latter is correct, the dispersal could either have been active or passive. South (1965) attempted to measure the ability of *Deroceras reticulatum* to actively disperse across arable land. He stated "The data suggest that *Agriolimax reticulatus* disperses little throughout its life. Boycott observed that molluscs are ill-adapted for dispersal, particularly with regard to recolonization of new habitats." One of the goals of the current study was to determine which of the above appear to have occurred.

METHODS AND MATERIALS

Almost all the field work was done by the senior author. During the summer of 1965 collections were made in Maine, New Brunswick, Quebec, Nova Scotia and Vermont; during the summer of 1966 collecting was done in Maine, New Brunswick, Quebec, Ontario, New York, Pennsylvania and portions of Connecticut and Massachusetts. Collecting was done in New England in the fall of 1965 and 1966, as well as in the spring of 1966. Robert L. Martin collected European slugs in Newfoundland during the summer of 1965.

To plan the field work for a particular state or province, a series of working circles, 50 mi. in radius, were drawn on a highway map. The primary consideration was to get adequate coverage for the region. The usual plan was to operate in each working circle for 3-6 days. The length of stay in each area depended upon the ecological complexity of the region, the preliminary collecting results, and the availability of access roads. In each working circle an attempt was made to search in as many of the following situations as possible: natural areas (woodlands, old fields), roadsides, rubbish dumps, picnic and camp grounds, cemetaries, gardens and yards, railroad rights-of-way, abandoned home sites and greenhouses. The emphasis, however, was on semi-cultivated and natural areas; relatively few gardens and private yards were visited.

The actual selection of collecting sites was generally made while driving along the route. Depending upon the nature of the habitat and the number of kinds of slugs present, anywhere from 10 min to 1½ hr were spent at each site. The average time was about 30 min. No attempt was made to adhere to a fixed minimum distance between sites. In general, however, sites were 10-20 mi apart, depending on the geography and ecological complexity of the region.

In certain instances, it seemed desirable to ascertain how deeply woodland slugs had penetrated into the wilds, away from camping areas, roadsides and other evidences of civilization. To do this a whole day was usually set aside in order to run a transect as far into the woods as time would permit, or until it appeared unlikely that any additional slugs would be found.

At woodland sites, slugs were sought under rocks, logs, loose bark, leaf litter and other debris. In fields and pastures a search was made under rocks, wood or other debris on the ground, as well as at the base of the grass or other plants. In picnic areas and camp grounds a search was made under trash barrels, fireplace rocks and firewood piles. In greenhouses, a search was made under debris on soil floors, on the underside of benches and under flowerpots. Where possible an attempt was made to search out-of-doors, under flats and boards in the immediate vicinity of the greenhouse. At each site all slug species found were collected. At sites in which a species was abundant an attempt was made to collect all color and pattern forms as well as all size classes. In all but a relatively few cases all slugs collected were immediately killed in 70% Ethanol. No attempt was made to relax specimens. In those few cases where the slugs were not killed they were returned alive for laboratory culture and study. Immediately after finishing at a site, field notes were made describing the precise locality, the dominant vegetation, the

species collected, and any other pertinent information.

All specimens were identified in the field on the basis of external morphology. The identification and the color form was entered in the field notes. In many cases, field identifications were later confirmed by dissection. This was especially true of the *Arion fasciatus* complex, certain color forms of *Deroceras laeve* and *D. reticulatum*, *D. caruanae*, and *Lehmannia valentiana*.

A few specimens of almost all species were sent for identification and comment to Dr. C. O. van Regteren Altena of the Rijksmuseum van Natuurlijke Historie, Leiden, Netherlands. Several lots of specimens of *Arion circumscriptus*, *A. silvaticus* and *A. subfuscus* were also sent for examination and comment to Dr. Henrik W. Walden of the Naturhistoriska Museet, Göteborg, Sweden.

Collections of European slugs in the Canadian National Museum, The American Museum of Natural History and the Yale University Peabody Museum were also examined.

RESULTS

Of the 770 sites visited (Fig. 1), 678 sites (88%), yielded at least 1 species of slug. Imported slugs were found in 510 sites (75% of all slug-yielding sites or 66% of the total number of sites visited). Chichester (1967) gives the precise locality, the habitat type and the species collected for each site. Fifteen species were collected, 12 of which were imported. Table 2 gives the number of sites yielding each species by state and province.

I. Geographical distribution

Arion subfuscus

This species proved to be much more widely distributed than previously reported (Fig. 2); it has also penetrated natural areas to such an extent that it has taken on the distributional pattern of a native species in many areas. In addition, 4 different color and pattern forms are present (Chichester & Getz, 1968b), each of which has its own distinct distributional pattern. Table 10 gives the characteristics of these color forms.

The distribution of the various color forms by province and state is as follows:

Quebec. The 2 principal populations occur near Gaspe' in the east, and Temiscaming in the west. The Gaspe' population belongs to Form 1, although it is more darkly pigmented than most other populations of this form. Based upon its homogeneity it was probably derived from a single importation in Gaspe'. The large number of other imported slugs and snails present in this area indicates, however, that the opportunity for importation must have existed more than once. The population is apparently a rather old one, judging from its wide distribution in natural areas in the vicinity of Cap des Rosiers and Gaspe'.

The Temiscaming population is homogeneous but somewhat intermediate between Forms 1 and 2. It was apparently introduced into Temiscaming from which it has spread westward into Ontario for some distance along Route 63, and eastward into the Kipawa Reserve. Paul Geissler collected specimens at several woodland sites in the heart of this remote reserve.

There are several scattered populations in the lower St. Lawrence River valley and the eastern townships. A small Form 4 population was found just above the New Hampshire border. Two small populations of Form 2 were found just above New York and Vermont from which they had undoubtedly dispersed. The other populations were generally assignable to Form 1; each appears to be very limited in its distribution.

New Brunswick. *A. subfuscus* has previously been reported only near the Nova Scotian border (Dimelow, 1962a). During this study it was found near Plaster Rock and in the Provincial Park

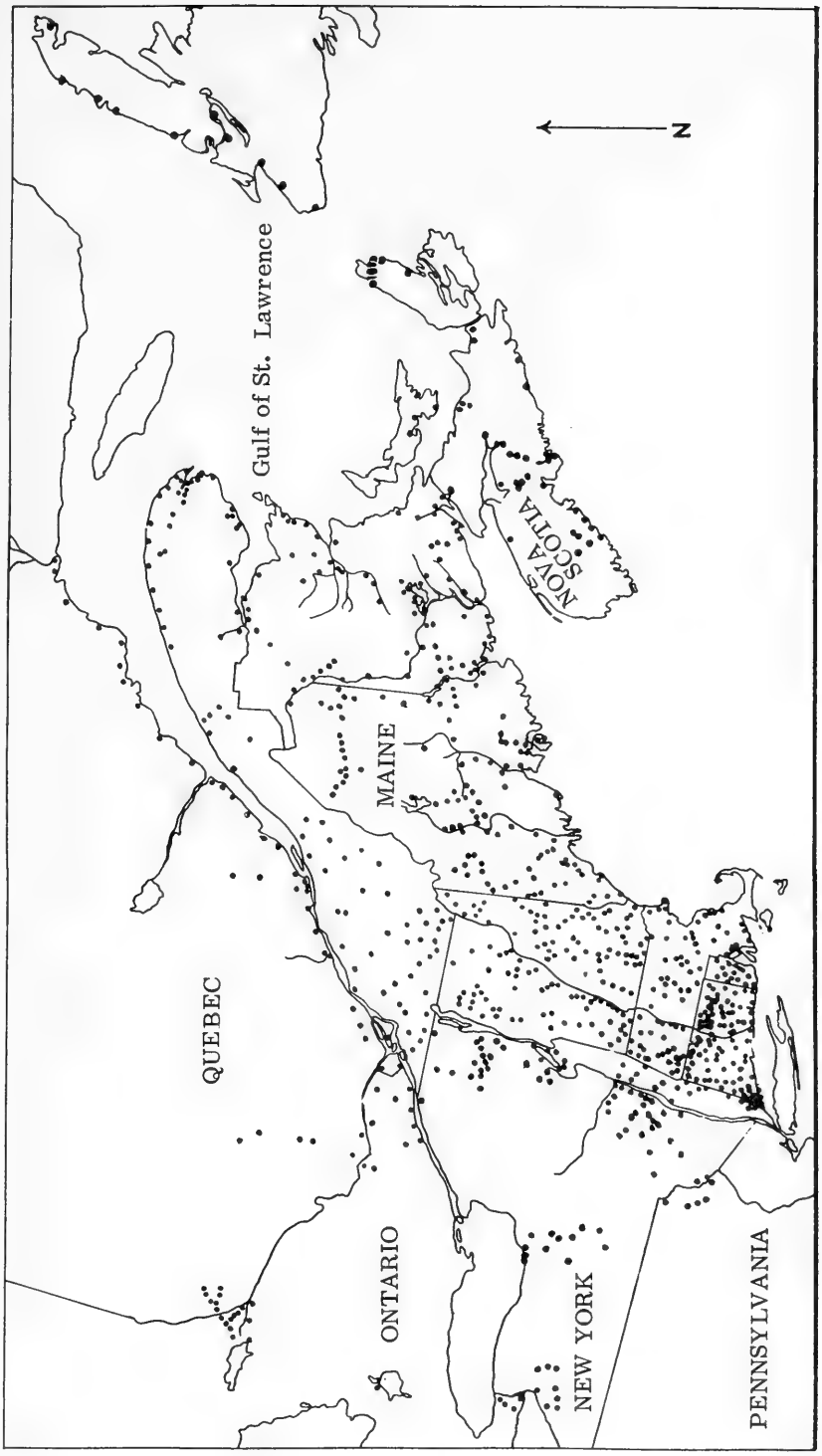

FIG. 1. Location of sites examined for slugs during the course of this study.

TABLE 2. Number of sites in each state and province at which slugs were found

Species	State or Province														
	Nova Scotia	New Brunswick	Quebec	Ontario	Newfoundland	Prince Edward Island	Maine	New Hampshire	Vermont	Massachusetts	Rhode Island	Connecticut	New York	Pennsylvania	% total sites visited (770)
Arion subfuscus	24	2	21	2*	7	..	39*	12	10*	12	..	19*	31	..	23
A. fasciatus	5	27	28	2	1	1	36	5	19*	18	2	23	32	2	26
A. circumscriptus	1	2	17	1	1	..	6	..	2	4	..	7	3	..	6
A. silvaticus	6	10	5	1	2	..	3	1	5	8	1	5	4	..	7
A. hortensis	1	..	1	..	..	..	..	..	..	1	..	6	..	..	1
A. intermedius	..	..	1	..	..	..	1*	2*	..	2*	2*	5*	2	..	2
A. ater	..	..	1	..	..	..	..	..	..	..	..	..	..	..	<1
Deroceras laeve	14	34	65	7	9	..	50	13	27	32	7	41	42	1	44
D. reticulatum	12	29	81	12	6	2	28	3	29*	13	1	19	43	2	36
D. caruanae	..	..	2‡	..	..	..	..	..	..	..	..	..	..	..	<1
Limax maximus	..	..	..	..	..	..	1	..	..	..	..	5	..	..	<1
Lehmannia valentiana	1‡	1‡	1‡	..	..	..	4*	..	..	2	..	6*	..	..	2
Milax sp.	1	..	..	..	..	..	..	..	..	..	..	..	..	..	<1
Pallifera dorsalis	3	16	14	4	..	..	17	8	11	12	2	8	20	2	15
Philomycus carolinianus	2	4	2	..	..	..	6	7	4	..	2	3	4	2	5

* New record for state or province

‡ New national record

.. Not found in state or province

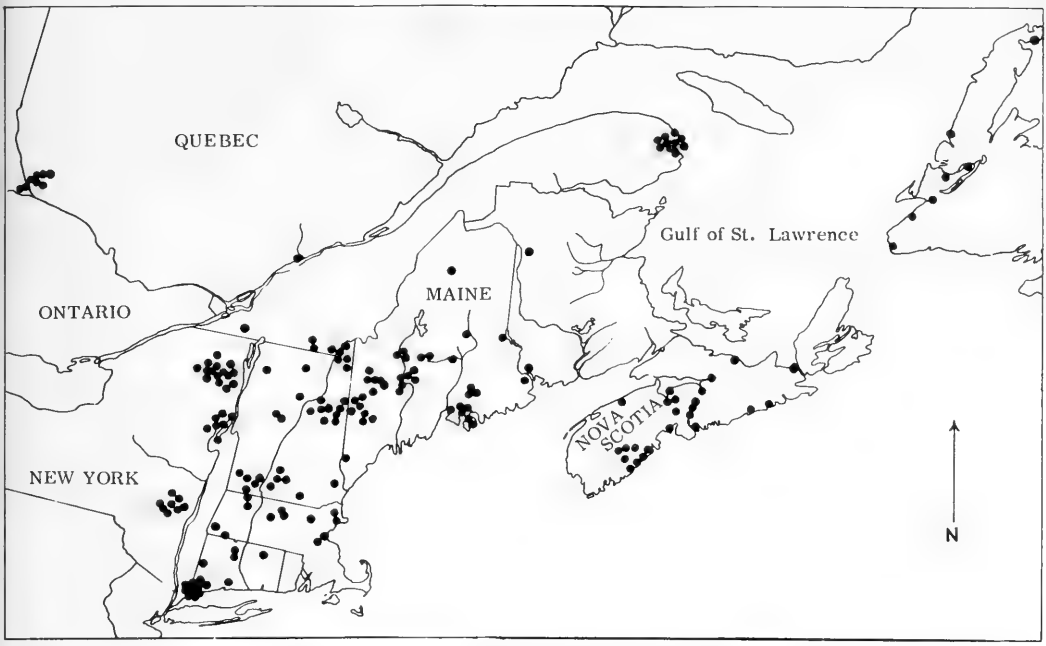

FIG. 2. Distribution of *Arion subfuscus* (all color forms) in northeastern North America.

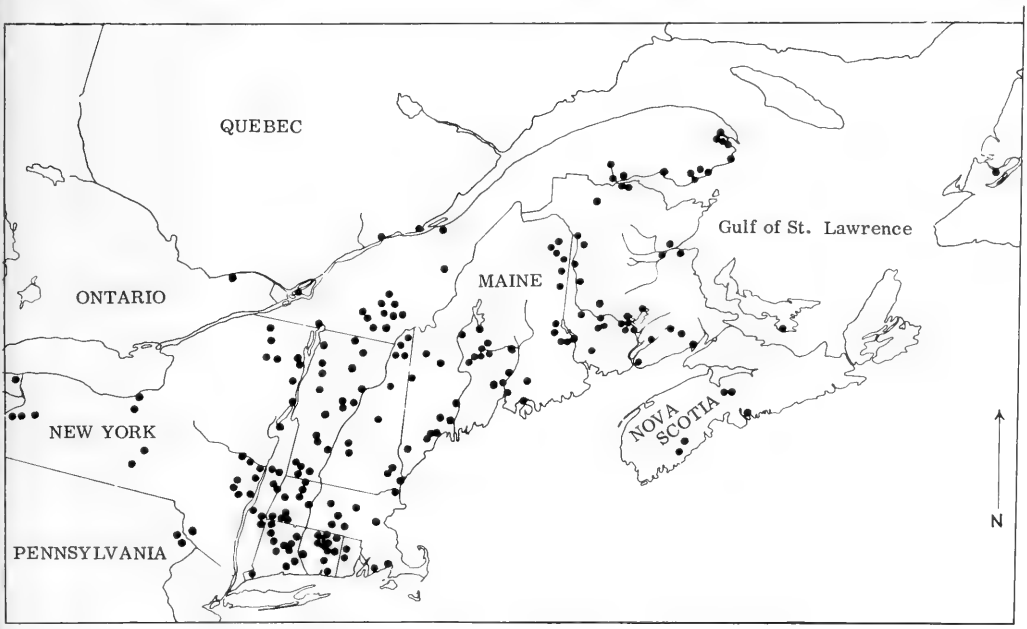

FIG. 3. Distribution of *Arion fasciatus* in northeastern North America.

at Oak Bay. The Oak Bay population consisted of Forms 1 and 4 along with several intergrading forms. It was, in fact, one of the most heterogeneous populations encountered. Based upon the failure to find it in any sites in the vicinity of Oak Bay, this population is apparently a local phenomenon. The Plaster Rock population was a homogeneous Form 2.

Nova Scotia. This species is widely distributed in Nova Scotia. All specimens were typical of Form 1. Based upon the distribution of other imported species as well as upon museum records the most likely portal of entry was Halifax. The Yale University Peabody Museum collection contained two specimens of *Arion subfuscus* (Form 1) from Halifax. These were collected by S. I. Smith sometime in the 1860's. The first published record for Nova Scotia was by Ord & Watts (1949). Most records for Cape Breton Island (Ord & Watts, 1949; MacMillan, 1954), are in the vicinity of the Canso Strait suggesting that at least a portion of the island population is simply an extension of the mainland population.

Newfoundland. Specimens of this species were assignable to Form 1, although they were darkly pigmented like the Gaspe' population. The southwestern records are the first for this species. The northwestern records confirm the findings of Brooks & Brooks (1940).

Maine. Both Forms 1 and 4, and intergrading forms, occur in southeastern Maine, east of the Penobscot River. They appear to be most abundantly distributed in the Ellsworth-Bar Harbor area which probably served as the portal of entry. Isolated populations near Grand Lake and Calais may have been derived from this rather heterogeneous population. Form 3 occurs throughout much of western Maine.

New Hampshire. Both Form 2 and 3 are abundant in the northern forested region. A small isolated population of Form 1 slugs was found in south-central New Hampshire, a few miles north of the Massachusetts border.

Vermont. Form 2 is abundant throughout forested regions. No other population was found.

Massachusetts. *Arion subfuscus* exists in numerous, widely scattered populations. Its distribution suggests that it has dispersed largely by passive means. Form 2 occurs in a few widely scattered sites in the western part of the state. Form 4 occurs in an essentially homogeneous population in Marlboro. Typical *A. subfuscus* (Form 1) was found in several scattered localities around the Boston area and elsewhere. The role of human activity in the accidental transport of slugs is nowhere more apparent than in Massachusetts and Connecticut. The pattern in these states is essentially quite different from northern New England and parts of New York State. In part, this is a reflection of the greater population density in southern New England which simply increases the probability of accidental transport. It also reflects the ecological differences which exist between southern and northern New England. Much of the woodland areas of southern New England are oak or oak-hickory forests in which *A. subfuscus* does not seem to occur. It may also reflect the fact that, until relatively recent times, much of southern New England was cultivated farm land. While *A. subfuscus* often occurs in such habitats, it is primarily a woodland slug.

Connecticut. Like Massachusetts, Connecticut has numerous scattered populations of *Arion subfuscus;* most of these are typically Form 1, although Form 2 occurs at several localities in Litchfield County in western Connecticut and Form 4 occurs in at least 1 locality in Hartford County. The most widely and abundantly distributed population (Form 1) occurs in Fairfield County, where it is common in natural and cultivated areas. This population may simply be part of a Greater New York City fauna. Form 1 occurs in Long Island (Jacobson, 1951; Westchester and

Putnam counties). It was found with the snail *Cepaea nemoralis* at one woodland site, and with the flatworm *Bipalium adventitium* at several localities in Greenwich. *C. nemoralis* was reported in the New York City area by Jacobson (1951). The flatworm was reported to be widely distributed in Westchester County by Klots (1960). Regardless of the portal of entry, the flower and shrub trade supported by the many large, long established estates in lower Fairfield County has undoubtedly been responsible for the dissemination of many imported slugs. European slugs were more abundant, both with respect to numbers of individuals and the number of species, in Greenwich than anywhere else in the Northeast.

New York. The 2 major populations (both Form 2) occur in the Catskills and Adirondacks. The populations of Westchester and Putnam counties have already been mentioned. No other population was found.

Pennsylvania. The principal reason for collecting in the northeast corner of Pennsylvania was to determine whether the Catskill population extended south into the Pocono Mountains. No slug of this species was found. Only a few sites were visited, however, owing to the very dry conditions that prevailed.

Arion fasciatus

This species is the most ubiquitous member of the genus. It was widely distributed in every state and province visited (Fig. 3). It generally does not penetrate very far into wild areas; however, it is by no means confined to cultivated areas. Unlike the previous species, *Arion fasciatus* shows little variation in pattern and color from one region to another. There does appear to be some slight variation owing either to diet or some other environmental factor. Slugs which were an overall gray when collected in the field soon acquired a distinctly yellowish-brown body color when reared on lettuce in the laboratory, as does Form 3 of *A. subfuscus* (Chichester & Getz, 1968a).

Arion circumscriptus

This species exists in 2 distinct color phases in the Northeast. The more widely distributed color phase is the typical black form described by Lohmander (1937). The other form is identical except that the black pigment is entirely replaced by reddish-brown pigment. This is even reflected internally in the brown spotted epiphallus and ovotestis. Presumably this dimorphism results from the presence of black melanin in the first form and brown melanin in the second. Extrapolating from Williamson's (1959) work a single pair of alleles may be responsible for this dimorphism. There is no intergrade between the 2 forms; an individual is either all black or all brown. The black form is rather widely distributed throughout the Northeast. The brown form appears to be largely restricted to southern New England where it may occur alone, or in company with the black form. This species is seldom present in large numbers at any one site. Figure 4 gives the distribution of this species in the Northeast.

Arion silvaticus

There appears to be but one form of the species in the Northeast; the species conforms very well to Lohmander's description, both externally and internally. There is usually no difficulty in distinguishing this species from the other 2 species in the complex. Like *Arion circumscriptus* it is widely, but sparsely, distributed (Fig. 4). It also is seldom present in large numbers in any one locality.

Arion hortensis

This species is probably more widely distributed than the records show. It was found in Quebec City, Halifax, near Boston, and in Mystic, Hartford and Greenwich (4 sites), Connecticut. A more thorough search of cultivated areas in the neighborhood of large cities

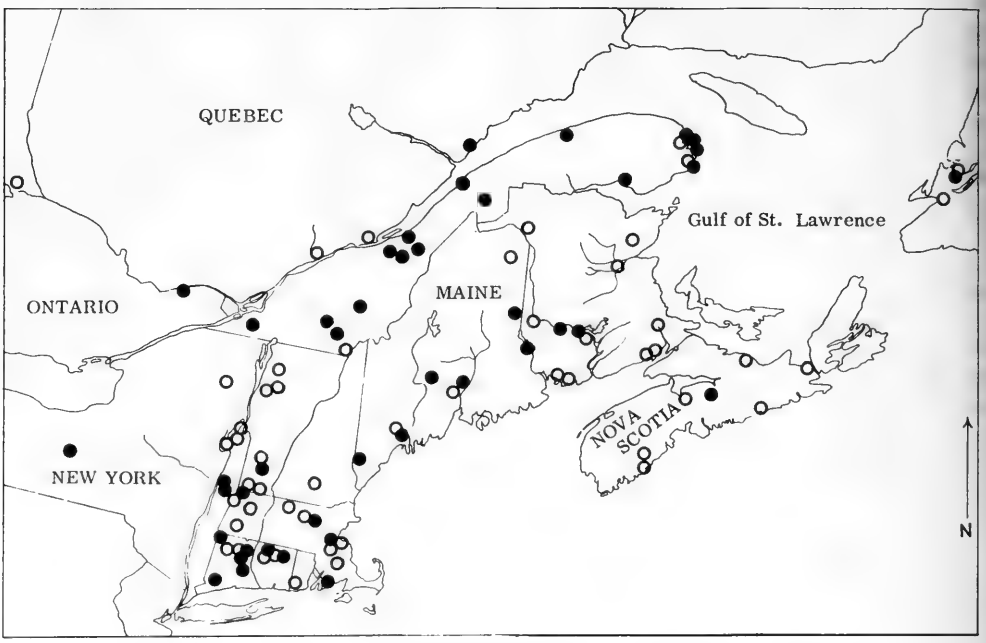

FIG. 4. Distribution of *Arion circumscriptus* (closed circles) and *A. silvaticus* (open circles) in northeastern North America.

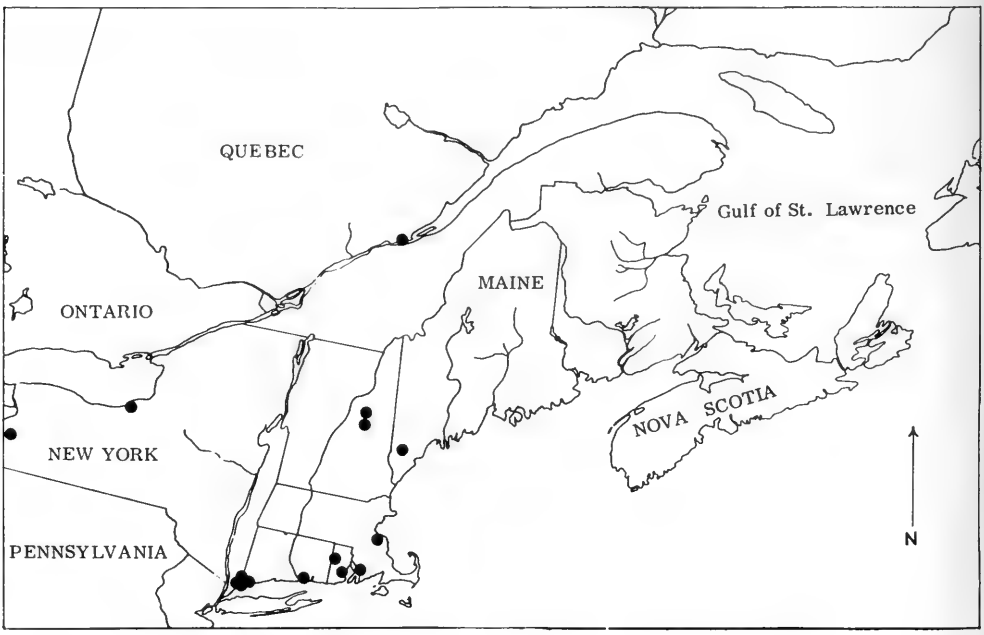

FIG. 5. Distribution of *Arion intermedius* in northeastern North America.

would probably yield many more records. Of the 9 sites at which this species was found only 2 were not directly associated with cultivated land. Of these, one was in close proximity to private homes; the other was in a deciduous woods in which 5 other species of European slugs and snails were found.

Arion intermedius

This species is much more widely distributed in the Northeast than previously suspected (Fig. 5). It occurs not only in cultivated areas where it is seldom abundant, but also in woodlands where it may be very abundant. The occurrence of *Arion intermedius* in woodlands not associated with cultivated areas suggests that this species may have had a much longer history in the Northeast than previous reports would suggest.

Arion ater

This species was collected at only one locality in the Northeast – Cape Bon Ami, Gaspe' Peninsula, P.Q. Two specimens (1 adult and 1 young banded individual) were found in the spruce-fir forest just below the face of a cliff, the same locality at which S. C. Ball (Pilsbry, 1948) found the species in 1938. It appears as if the species has not dispersed very far. None was found at any of the other 11 sites visited in the vicinity of Gaspe' and Cap des Rosiers. By comparison, *Arion subfuscus* was found at 8 of these sites, as well as at Cape Bon Ami.

Deroceras laeve

This native species is the most widely distributed slug in the Northeast. It occurs in wild areas and cultivated habitats of almost every description. It is externally dimorphic with one form having the dorsum darkly reticulated or speckled; the other form is uniformly colored. It is also internally dimorphic in that some individuals are euphallic, others are aphallic. Although in general there does not appear to be any correlation between the 2 types of dimorphisms, certain large, unreticulated greenhouse forms seem almost always to be aphallic.

Deroceras reticulatum

This is the most widely distributed European species. It achieves very large population densities in fields and certain cultivated areas. Like *Arion fasciatus* it has an essentially continuous distribution throughout most of the Northeast. It is quite variable with respect to color throughout its range; body color varies from white, through tan and gray, to black.

Deroceras caruanae

The 2 greenhouse records for Quebec (Sherbrooke and Quebec City) are new records for eastern North America. The Sherbrooke greenhouse yielded 2 specimens, the Quebec City greenhouse yielded 4 specimens.

Limax maximus

Undoubtedly, a greater emphasis on collecting in cultivated lands, especially in urban areas, would have yielded many more locality records. The only record for this species in a natural area, well removed from cultivated lands, is in Acadia National Park, near Bar Harbor, Maine. Other records, all in Connecticut, are for Greenwich, New Britain (2 sites), North Branford and Meriden.

Lehmannia valentiana

This species is present in most commercial greenhouses. Of the 20 greenhouses (private, commercial and governmental) visited 14 yielded this species. These were located in Halifax, Newcastle and Sherbrook, Canada; Auburn, Presque Isle, Gorham and Ellsworth, Maine; Worchester, Massachusetts; Greenwich, Bridgeport, Cromwell, Stafford Springs and Storrs, Connecticut. It is often present in large numbers. In only 1 case was this species found away from a greenhouse or its environs. In this single instance, 1 specimen was found in a backyard of a suburban home near Boston. This same

backyard also yielded 6 other imported species of Mollusks.

Milax sp.

This genus was found in only 1 locality, the Public Gardens Greenhouse in Halifax, Nova Scotia. The slugs were very abundant, but all specimens collected were immature, making species identification uncertain. Dr. Altena, who examined some of these specimens, thought they might be *Milax gagates*, but couldn't be positive because of their immaturity.

Pallifera dorsalis

This native species is widely distributed. It displays considerable variation in some characters, such as the spotting pattern on the mantle and the rust-red coloration of the anterior portion of the foot.

Philomycus carolinianus

This native species is a locally scarce, but widely distributed, woodland slug. It was found in only 36 sites. The species shows very little color and pattern variation in the Northeast.

Limax marginatus

This species was not found in any state or province. The failure to collect it at any of the dozen sites in western Newfoundland may indicate that the species has not yet dispersed to the west coast of the island.

Limax flavus

No specimen of this species was found. This is surprising in view of the many early records for this species in eastern United States.

State and provincial records

Table 3 gives the overall collecting results for each state and province. Columns 4 and 5 provide a rough indication of how well imported slugs have managed to penetrate a region. Column 6 is essentially the probability of encountering an imported slug in a given state or province.

During the summers of 1965 and 1966 much of the northeast was in a prolonged drought. The relatively low percentage of sites yielding slugs in New Brunswick and Ontario is undoubtedly related, in part, to the very dry conditions in these areas.

II. Ecological distribution

The 770 collection sites were grouped into 12 different categories or habitat types. Establishment of these categories involved consideration of a combination of ecological and land-use factors. The primary concern, however, was what habitats slugs actually select as well as what environmental situations reflect, or promote, their passive dispersal by means of accidental transport.

About 38% of the collection sites were woodlands; 33% were basically ecotonal (roadside margins and deciduous thickets); 22% were fields; and about 7% were cultivated or highly modified areas (Table 4).

The habitat categories are as follows:

Coniferous woods. The 2 principal types of coniferous woods in the Northeast are the spruce-fir forests of northern New England and Canada, and the pine woodlots and groves scattered throughout the region. In the first type only *Arion subfuscus* achieves large population densities, although all 3 native species are found there. In the latter type, both native and imported species are scarce.

Mixed woods. Most combinations of coniferous and deciduous trees in the Northeast are more productive of slugs than are coniferous stands alone; only oak-pine and oak-hemlock have few slugs. In addition to *Arion subfuscus* and the 3 native species, *A. fasciatus*, *A. silvaticus* and *A. circumscriptus* may also be present in small numbers in mixed deciduous-coniferous woods.

Deciduous woods. Only oak and oak-hickory stands are less productive of slugs than either coniferous or mixed

TABLE 3. Frequency of occurrence of imported slugs by state and province

State or Province	1*	2	3	4	5	6
Quebec	130	126	99%	102	81%	78%
New Brunswick	80	63	79%	46	73%	58%
Nova Scotia	39	36	92%	31	86%	79%
Prince Edward Island	3	2	67%	2	100%	67%
Newfoundland	12	12	100%	10	83%	83%
Ontario	27	19	70%	15	79%	56%
Maine	102	98	96%	74	76%	72%
New Hampshire	43	35	81%	18	52%	42%
Vermont	60	53	88%	41	77%	68%
Massachusetts	73	56	77%	35	63%	48%
Connecticut	94	76	81%	55	72%	59%
Rhode Island	15	11	73%	5	45%	33%
New York	87	86	98%	74	86	85%
Pennsylvania	5	5	100%	2	40%	40%

*1 Total number of sites visited
2 Number of sites yielding slugs
3 Percentage of sites yielding slugs (both native and imported)
4 Number of sites yielding imported slugs
5 Percentage of slug sites yielding imported slugs $\left(\frac{\text{Column 4}}{\text{Column 3}} \times 100\right)$
6 Percentage of total sites yielding imported slugs $\left(\frac{\text{Column 4}}{\text{Column 1}} \times 100\right)$

TABLE 4. Number of sites sampled in each habitat type during the present study. Note: some sites sampled contained more than one habitat type and are thus included more than once

Habitat Category	Number	Percentage of the total
Coniferous woods	75	9
Mixed woods	131	15
Deciduous woods	125	14
Deciduous thickets	37	3
Fields	194	22
Dumps	25	3
Roadside margins	255	30
Nurseries	2	< 1
Estates	3	< 1
Gardens	3	< 1
Greenhouses	20	2
Cemeteries	9	1

woods. The optimum woodlot is one composed of maple or maple and ash. If there is ample leaf and twig litter and logs, *Arion fasciatus*, *A. circumscriptus* and *A. silvaticus* all achieve large population densities; *A. subfuscus* and the native species also are abundant. On occasion, *Deroceras reticulatum* may also be present in small numbers.

Deciduous thickets. This category was included to cover ecotonal situations in which a clump of small deciduous trees or shrubs was interposed between 2 other habitats, such as a field and a coniferous woods, or in which the trees or shrubs formed a fence row. Such areas make it possible for primarily woodland slugs to penetrate into fields and cultivated areas by providing cover during adverse periods.

Roadside margins. This category includes the grassy ditch adjacent to the road; the grass, forbs, and young deciduous trees next to the ditch; and the immediately adjacent margin of the woods. In effect, a grassy ditch is nothing more than a very elongated field; most of the reports of *Deroceras reticulatum* under this category actually refer to this grassy margin. The grass-forbs-deciduous tree band is an ecotone between the ditch and the woods. In spruce-fir country, *Arion fasciatus* and *D. reticulatum* are often present in the grass and ecotonal strip, but usually not in the coniferous woods.

Fields. This term includes a roadside ditch when it was adjacent to a pasture or field, an abandoned field, log storage areas, pastures and abandoned orchards. Both *Deroceras laeve* and *D. reticulatum* are very common; *D. reticulatum* in particular achieves very high population densities in all of these areas. *Arion fasciatus* is also commonly present, but usually not as abundantly as *D. reticulatum*.

Dumps. Both municipal dumps and illicit roadside dumping areas provide an abundance of decaying matter which may serve as food, as well as an abundance of hiding places. Dumps do not appear to be important in the passive dispersal of slugs, but often allow artifically high population densities to be achieved.

Nurseries. Only two nurseries were visited; both yielded *Arion subfuscus*. One of them also yielded *Lehmannia valentiana* (in the nursery greenhouse), *A. hortensis*, *A. intermedius* and *Deroceras reticulatum*. There were several *A. subfuscus* in and under flats of *Pachysandra* waiting to be loaded on a delivery truck.

Estates. Restricted to those highly cultivated park-like private estates in which several different habitats were present. Only 3 estates in southwestern Connecticut were visited. All 3 yielded imported species in large numbers including *Arion subfuscus*, *A. hortensis*, *A. intermedius* and *Deroceras reticulatum*.

Gardens. Only 3 small private gardens were visited; all 3 yielded *Arion fasciatus;* 2 yielded *Deroceras reticulatum*. One yielded *Limax maximus*.

Greenhouses. Twenty greenhouses (commercial, private and governmental) were visited throughout the region. A large portion of the greenhouse fauna is remarkably constant from one region to another; it consists of *Lehmannia valentiana*, *Deroceras reticulatum*, *D. laeve* and snails such as *Oxychilus draparnaldi*, *O. cellarius*, and *Zonitoides arboreus*. This implies that there is a considerable amount of accidental inter-greenhouse transfer of mollusks. The remaining portion of the greenhouse fauna often seems to reflect the local situation; the presence of *Arion fasciatus* or *A. subfuscus* in a greenhouse usually seems to reflect their abundance in the surrounding countryside.

Cemeteries. Nine cemeteries were visited throughout the region. In general, slugs present in a cemetery were also widely distributed in the surrounding countryside. Since most floral decorations brought into cemeteries consist of cut flowers, rather than potted plants (although potted plants are sometimes

placed on graves), it would seem unlikely that very many slugs disperse in this manner.

Habitats of the species

Table 5 gives the number of times each species was encountered in each habitat type. Table 6 gives the percentage of the encounters which occurred in each habitat type, so as to facilitate comparisons between the more widely distributed species. Table 7 groups all of the habitat categories under 4 major headings as follows: woodlands, which includes coniferous, mixed and deciduous woods; ecotones, which includes roadside margins and deciduous thickets; fields, as a separate category; and cultivated areas, which includes greenhouses, gardens, estates, nurseries, cemeteries and dumps. The results given in Tables 6 and 7 generally agree with accounts published by European workers on the ecological distribution of European species. *Deroceras reticulatum* is even more of a field slug than Tables 6 and 7 indicate; most encounters recorded under "roadside margins" (and ecotones) were in the grassy ditch adjacent to highways.

Interspecific association

Another way of extracting ecological information is to examine species associations on the assumption that species with similar ecological requirements occur together more frequently than do species with widely different ecological requirements. Table 8 shows the number of times that selected species occurred together (excluding greenhouse records). Table 9 gives an index of association for selected species pairs, which is simply the number of times (expressed as a percentage) each species pair occurred out of the total number of times in which it could possibly have occurred.

As one might expect *Deroceras reticulatum* (a field slug) occurred more frequently with *Arion fasciatus* (basically a ecotonal slug) than with *A. subfuscus*, *Pallifera dorsalis* or *Philomycus carolinianus* (all basically woodland slugs). *Philomycus carolinianus* is more frequently found together with *Pallifera dorsalis* than with *A. subfuscus*. This, in part, results from the fact that both native woodland species occur in oak or oak-hickory forests where *A. subfuscus* apparently does not occur. It also reflects the fact that *A. subfuscus* is an imported species and, hence, not as widely distributed in some woodland areas as the native species (e.g., New Brunswick). That *A. subfuscus* shows no strong tendency to associate with any other species also suggests that its ecological requirements may be rather different. The tendency for *D. laeve* to occur frequently with most other species testifies to its ecological versatility.

DISCUSSION

I. The significance of the color forms of Arion subfuscus

Attempts to describe color phases of *Arion subfuscus* in Europe have been frustrated by the almost endless variations that seem to occur within the species. The same problem existed with *A. ater;* however, Williamson (1959) has provided a genetic basis for understanding the variations that occur within that species. It is apparent when one analyzes the nature of the color variations in *A. subfuscus* that a similar, but perhaps somewhat more complex, genetic explanation would account for the various color forms of this species as well. It is reasonable to assume that the polymorphism that occurs in this species throughout most of Europe results from the alleles controlling some aspect of color or pattern being in general circulation and constantly undergoing recombination.

In North America *Arion subfuscus* is also polymorphic, but in a different way than apparently occurs through most of Europe. In the Northeast, one frequently finds an entire population, sometimes

TABLE 5. Number of sites within each habitat type that each species of slug was collected

Species	Habitat Type											
	Coniferous woods	Mixed woods	Deciduous woods	Deciduous thicket	Roadside margins	Fields	Dumps	Nurseries	Estates	Gardens	Greenhouses	Cemeteries
Arion subfuscus	32	38	36	4	37	24	6	2	3	..	2	..
A. fasciatus	10	30	34	6	52	57	12	..	..	3	7	2
A. circumscriptus	3	4	9	3	16	8	1	..	..	..	..	..
A. silvaticus	1	1	16	1	9	19	1	..	..	..	1	1
A. hortensis	..	..	2	2	1	..	..	1	2	..	1	..
A. intermedius	..	1	5	2	3	1	1	1	1	..	..	..
A. ater	1	..	..	..	..	..	..	..	..	..	..	..
Deroceras laeve	39	47	52	8	98	69	14	2	2	1	18	3
D. reticulatum	14	19	34	7	75	108	13	1	2	3	17	1
D. caruanae	..	..	..	..	..	..	..	..	..	..	2	..
Limax maximus	..	..	1	1	..	2	..	..	..	1	..	..
Lehmannia valentiana	..	..	1	..	..	1	..	..	..	..	14	..
Milax sp.	..	..	..	..	..	..	..	..	..	..	1	..
Pallifera dorsalis	20	32	29	..	26	9	1	..	..	..	..	1
Philomycus carolinianus	4	15	13	..	44	1	..	..	..	..	..	..

TABLE 6. Percentage of sites within each habitat type selected species were collected

Species	Habitat											
	Coniferous woods	Mixed woods	Deciduous woods	Deciduous thicket	Roadside margins	Fields	Dumps	Nurseries	Estates	Gardens	Greenhouses	Cemeteries
Arion subfuscus	17	21	20	2	20	13	3	1	2	..	1	..
A. fasciatus	5	14	16	3	24	27	6	..	..	1	3	1
A. circumscriptus	7	9	20	7	36	18	2	..	..	..	..	..
A. silvaticus	2	2	32	2	18	38	2	..	..	..	2	2
Deroceras laeve	11	13	15	2	28	20	4	<1	<1	<1	5	4
D. reticulatum	5	7	12	2	28	37	4	<1	<1	<1	6	1
Pallifera dorsalis	17	27	25	..	22	8	4	..	..	..	..	<1
Philomycus carolinianus	11	41	35	..	11	3	..	..	..	..	..	..

TABLE 7. Frequency of occurrence (percentage) of selected species in major habitat types*

Species	Major Habitat Types			
	Woodlands	Ecotones	Fields	Cultivated areas
Arion subfuscus	58	22	13	7
A. fasciatus	35	27	27	11
A. circumscriptus	36	43	18	2
A. silvaticus	36	20	38	6
Deroceras laeve	39	30	20	11
D. reticulatum	23	28	37	13
Pallifera dorsalis	69	22	8	1
Philomycus carolinianus	87	11	2	..

*See text for definitions of major habitat types.

TABLE 8. Number of times selected species were collected at the same site

	D. reticulatum	*A. fasciatus*	*A. subfuscus*	*D. laeve*	*P. dorsalis*	*P. carolinianus*
Deroceras reticulatum	-	101	40	108	27	7
Arion fasciatus	101	-	44	87	21	5
Arion subfuscus	40	44	-	58	30	9
Deroceras laeve	108	87	58	-	52	8
Pallifera dorsalis	27	21	30	52	-	18
Philomycus carolinianus	7	5	9	8	18	-

TABLE 9. Frequency of association (percentage) of selected species pairs*

	D. reticulatum	*A. fasciatus*	*A. subfuscus*	*D. laeve*	*P. dorsalis*	*P. carolinianus*
Deroceras reticulatum	X	50	22	39	23	19
Arion fasciatus	50	X	25	43	18	14
Arion subfuscus	22	25	X	32	26	25
Deroceras laeve	39	43	32	X	44	22
Pallifera dorsalis	23	18	26	44	X	50
Philomycus carolinianus	19	14	25	22	50	X

*Based on data in Table 8.

extending over a wide area, in which all individuals are almost identical in regard to color form. These color form populations occur over relatively large geographic areas and in a variety of ecological situations. It does not appear, therefore, that their distribution reflects different ecological tolerances or that localized conditions of vegetation or soil type and composition affects the colors developed by the slugs. In addition, laboratory colonies reared under standardized conditions retained the same color pattern as the wild types.

The most reasonable explanation for the occurrence of a completely homogeneous population in this species is that the population arose from a single (homozygous) founder stock. The significance of this phenomenon is that many populations are very precisely labeled allowing one to determine their center of origin and the extent of their dispersion. In the future, knowing the present day distribution, one should also be able to follow the dispersal of many of these populations. The present distribution of the various color forms clearly indicates *Arion subfuscus* has been introduced several times.

One of the best examples of a homogeneous population that arose from one separate and distinct importation is represented by Form 3. This form occurs in the western half of Maine where it appears to be the only form of the species present. It is widely distributed, especially in natural areas, suggesting that it is not a recent import. It also occurs through the northern half of New Hampshire, often in company with Form 2, with which it apparently interbreeds (Getz, 1962b). Getz obtained both color forms, without intergradation, from the same egg mass. Field observations of populations in which both forms are present also reveal no intergradation. The failure to produce intergrades may result from the specific combination of alleles (linkage groups) present in both color forms. Form 3 is also found in Quebec just north of the New Hampshire boundary. The only other locality at which this form was found was in North Branford, Connecticut, where a single specimen was found. It may have been transported south from either Maine or New Hampshire in plant materials. The portal of entry for this form was probably in southwestern Maine since it is most widely distributed there. It appears to be spreading westward into New Hampshire and northward into the remote regions of Maine, where it has been found as far north as Churchill Lake in northern Piscataquis County. It was not found in coastal Maine or New Hampshire, suggesting that it may have been introduced into an inland locality.

Populations belonging to Form 2 may also be derived from a single introduction, but the evidence is less definitive. Form 2 occurs very abundantly in the Catskill and Adirondack Mountains of New York. It is abundant, but not quite so widely distributed, in the forested regions of Vermont and New Hampshire. It also occurs in western Connecticut and Massachusetts in widely scattered localities. Both New York populations are essentially identical in appearance; it is likely that these 2 populations resulted from a single introduction although it is not possible to say at this time which population was established first. Both Vermont and New Hampshire populations were also probably derived from a single introduction. The question is whether or not the Vermont and New Hampshire stock was derived from either of the New York populations. It seems likely that they were. The argument in favor of this interpretation is that it seems unlikely that 2 essentially identical color forms, out of the many possibilities created by intergradation in Europe, would be introduced into adjacent states (i.e., New York and Vermont). Complicating the matter is the presence of this form at one locality in western New Brunswick. This small, isolated population was found a few

TABLE 10. Characteristics of the color forms of *Arion subfuscus*

Color Characteristic	Color Forms			
	Form 1	Form 2	Form 3	Form 4
Dorsal Melanin[1]	Solid	Streaked	Absent	Absent (usually)
Lateral Bands[2]	Present	Present	Absent	Faintly present
Reticulated Sides[3]	No	Yes	Yes	No
Melanin Color[4]	Usually black	Black	Black	Brown or black
Intensity of Pigmentation[5]	Moderate	Very intense	Very intense	Faint
Red Pigment[6]	Yellow	Orange or yellow	Yellow (usually)	Yellow

[1]The dorsum is either darkly and uniformly pigmented so as to form a wide median band between the lateral bands; or it is darkly pigmented but streaked in such a fashion as to form 2 longitudinal bands dorsal to the usual lateral bands; or the dorsum may be completely unpigmented; or it may be very faintly and uniformly pigmented.

[2]Lateral bands may be faintly or distinctly present; or they may be virtually absent. In the color form in which bands are virtually absent (Form 3) a very short vestige of the bands is present on the extreme posterior end of the animal.

[3]The sides of the body may be unpigmented, or darkly reticulated.

[4]The pigment, so far referred to, is assumed to be melanin. It may be black or brown. Both colors are apparently not present in the same individual.

[5]The dark color may be very intense, moderately intense, or faint.

[6]Red pigment, which is either rufine or rufine-like, is present in the mucous glands and is shed with the slime. It varies in color from reddish-orange to very pale yellow (differences are presumably due to pigment concentration effects). It gives the animal an overall red or yellow appearance. The characteristics of the 4 color phases are given in Table 10.

miles west of Plaster Rock. The site was a cutover spruce-fir forest, far from homes and cultivated lands. The only signs of civilization, other than the fact that timber had been harvested a few years earlier, was the road and a nearby Canadian National Railway right-of-way. The slugs were present in great numbers on both sides of the road. They were abundant for at least 200 yards. No other specimen of this or any other color form were found anywhere near this locality. Until that part of New Brunswick is more thoroughly examined the significance of this population will remain in doubt. The small scattered populations in southern New England give every indication of being transplants from either New York or northern New England.

All other populations of this species in the Northeast belong to color forms 1 and 4. Many of the populations assigned to either form are quite heterogeneous and often reflect a great deal of mixing. Other populations of Form 1 are quite homogeneous. It is doubtful whether Form 4 ever occurs in homogeneous populations in the same sense that this term has been used previously. However, there are a few populations in which all individuals fall within the limits described for Form 4.

An analysis based upon only 770 sites in an area as large as the entire Northeast is undoubtedly subject to error; certainly many small populations were missed. However, the broad outlines appear to be correct, even if some details are in error. Except in rare instances, the populations discovered during the course of this study were probably old, well established ones. It is apparent that this species was introduced very early. Using the relative abundance of the species in Nova Scotia, where we know it was present in the 1860's, as a crude sort of guide, it seems likely that this species was already present in several other localities by the middle of the last century. It is surprising that there are not more records of this species because it is a large and conspicuous slug. It may be that, because it is more a woodland slug than the other 2 widely distributed species, it was overlooked.

II. Distributional patterns of other species

Unfortunately, it is not possible to perform the same type of analysis of the distribution of other widely distributed species. They are not "labeled" so conveniently as *A. subfuscus*. However, there is no reason to believe that their histories are very different in broad outline, though they may differ in many details.

Arion fasciatus and *Deroceras reticulatum*. Both species are so widely distributed that it is no longer possible to identify portals of entry. Undoubtedly both have been introduced many times in both Canada and the United States. There are probably several factors that have contributed to their ability to disperse so widely, but a primary reason is that they occur in large numbers in those habitats in which the chances for accidental transport is greater.

Arion circumscriptus and *A. silvaticus*. Both species are widely distributed, but less frequently encountered than *A. fasciatus*. This is related to the fact that both species usually exist in very small numbers at any one locality. As a result, the probability of accidental transport is reduced. Since the probability of detection at any one site is also low, it is very likely that both species are more widely distributed than this study indicates.

There does not appear to be any common pattern in the distribution of these 2 members of the *A. fasciatus* species complex. Each has apparently had its own unique importation and dispersion history. *Arion circumscriptus* is more widely distributed in Quebec; it was the only species in the complex found along the northshore of the Upper St. Lawrence. *Arion silvaticus*, on the other

hand, is almost uniformly abundant by state and province. Both species are apparently at the stage where they are established at numerous secondary centers from which their dispersal is just now beginning.

Arion intermedius. This species has probably been present in the Northeast for a long time. Although in several instances it was associated with cultivated areas, in other cases it was found in natural areas, existing in small, apparently isolated, populations. It is probably much more widely distributed in coastal New England and western New York than this study reveals. Unlike *A. hortensis* which is restricted to cultivated areas, this species shows signs of eventually becoming widely distributed in natural areas. It apparently disperses at a very slow rate, however.

Arion hortensis and *Limax maximus*. Both species have been introduced several times and have managed to reach numerous secondary centers in cultivated areas, to which they have largely remained restricted.

Other species. The other species are so rare or so restricted in their distribution that no further discussion is warranted at this time.

III. A Model of importation and dispersion of European slugs

From an analysis of the probable manner in which the present distributional pattern of *Arion subfuscus* was established it is possible to suggest certain stages through which imported species have passed, or are passing, in the process of becoming naturalized. These stages are:

Importation. Obviously the first step was to gain entry. Most species have achieved introduction on more than one occasion; *Arion subfuscus* has been introduced more than half a dozen times.

Local establishment. Once introduced, the species had to establish itself in the vicinity of the portal of entry which usually was an urban area. The more ecologically versatile the species, the better were the chances for establishment. For example, *A. subfuscus*, though it is primarily a woodland slug, often establishes very large populations in highly cultivated areas.

Transport to secondary centers. Transplants from the portal population were unintentionally transported to outlying areas. The frequency with which a species was disseminated was probably directly related to the size of the population in the vicinity of the portal of entry, to the ecological versatility of the species, and to the nature of the portal of entry itself (i.e., the nature of its commerce).

Establishment in secondary centers. The species faced basically the same problems as it did in the portal of entry except that many of these secondary centers were not urban areas. Those species which are not normally restricted to urban areas, were often provided with the opportunity to exploit suitable habitats. It is possible that the large, well established populations of *Arion subfuscus* in the Catskill and Adirondack Mountains represent secondary populations that flourished while the primary center in some urban center either disappeared or remained insignificant.

Active dispersal. This undoubtedly occurred at the portal of entry as well as at the secondary center. The Gaspe' population provides us with an example of active dispersal from a primary center; this is undoubtedly related to the suitable woodland habitat that exists in the vicinity of Gaspe'. The spread of *Arion subfuscus* throughout the Catskills and Adirondacks may be examples of active dispersion from a secondary center. Other widely distributed species also demonstrate a considerable ability to disperse without the direct participation of man. For example, both *Deroceras reticulatum* and *A. fasciatus* are

frequently found in roadside margins adjacent to forest lands, often miles from any cultivated areas. The most reasonable explanation is that these species disperse in the moist, grassy ditch and the narrow ecotonal strip between the ditch and the woods. This dispersal ability of slugs has been denied by several authors including Boycott (1934) and South (1965).

Naturalization. In this stage the species achieves an essentially continuous distribution in suitable habitats throughout the region. This is achieved when populations spreading from many secondary centers finally overlap. An added qualification is that the species should be able to maintain stable populations that reflect an accommodation between itself and the community into which it has dispersed. On the basis of the first criterion two species, *Arion fasciatus* and *Deroceras reticulatum*, have almost reached this stage; one species, *A. subfuscus*, is approaching this stage. On the basis of the second criterion, it is not yet possible to comment. The matter requires further study.

IV. Probable mechanisms of importation and dispersal

This study set out to test the hypothesis that slugs have been introduced in ballast dumped on shore by European sailing ships. The results do not support the hypothesis. While ballast introductions cannot be completely ruled out in a few places such as Gaspe', Nova Scotia and Newfoundland, the most important means of gaining entry was with imported plant materials. There have been too many introductions, many of them at inland localities, to support ballast transport as the sole or major source of imported slugs. The best explanation for coastal slug populations is that most plant imports were directed to the larger, long-established cities, many of which are on the sea coast.

Passive dispersal within the Northeast has also probably depended largely upon transport with nursery and greenhouse plants, although transport with soil, logs and other materials has undoubtedly also occurred.

As stressed earlier, active dispersal has been more important than anyone realized. It has certainly been important in the case of *A. subfuscus;* there is no other reasonable explanation for its wide distribution in natural areas. It has probably also been an important factor in the spread of *D. reticulatum* and *A. fasciatus*, especially in their ability to bypass barriers such as coniferous forest (in which neither species is ever very abundant), or dry areas.

V. Significance of imported slugs

Most of the interest in imported slugs to date has been from an economic point of view, because of the tendency for a few species to establish large populations in gardens, greenhouses and fields. Field slugs are undoubtedly of considerable economic importance, especially in dairy and potato growing regions in the Northeast. However, the losses due to depredation by them probably will not increase very much beyond present levels, except for occasional years in which more favorable than normal moisture and temperature conditions prevail.

The possible problems associated with *Arion subfuscus* are another matter. There is no obvious barrier that will prevent the ultimate spread of this species throughout much of the forested regions of northern North America. Where it now occurs in forested regions, it achieves high population densities. Of all the imported species, *A. subfuscus* has the potential for having the greatest impact upon natural communities. It is essentially filling a void in an otherwise mollusk-poor region. It constitutes an abundant, new food supply for small mammals and other animals in many parts of the Northeast. While there is no evidence as yet that this species is important in the transmission of animal or plant parasites, its presence in large

numbers in natural areas where terrestrial mollusks have not previously been abundant should be of some concern to wildlife parasitologists and plant pathologists.

For many reasons *Arion subfuscus* has proved to be the most interesting and, potentially, the most important introduced slug. It should provide the basis for additional studies along both basic and applied lines.

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RÉSUMÉ

LA ZOOGÉOGRAPHIE ET L'ECOLOGIE
DES ARIONIDÉS ET LIMACIDÉS INTRODUITS DANS LA RÉGION
NORD-EST DE L'AMÉRIQUE DU NORD

L. F. Chichester et L. L. Getz

On a procédé à l'étude de la distribution géographique et écologique de 12 espèces d'Arionidés et de Limacidés, importés dans le Nord-Est de l'Amérique du Nord. Au total 770 biotopes ont été visités dans 8 états et 5 provinces; parmi ceux-ci 550 biotopes comportaient au moins un espèce importée. Trois espèces (*Deroceras reticulatum*, *Arion fasciatus* et *A. subfuscus*) ont été trouvées en grande abondance. Le statut des 3 composantes morphologiques du complexe de *A. fasciatus* est discuté; ces composantes sont traitées comme des espèces distinctes au long de l'exposé. La découverte de nouvelles localités a eu lieu pour *A. intermedius*, *A. hortensis*, *A. subfuscus* et *D. caruanae*.

Les 3 espèces les plus largement représentées montrent des préférences écologiques différentes. *Arion subfuscus* est essentiellement une limace des bois, *D. reticulatum* une limace des champs. *Arion fasciatus* est plus abondante en situations "écotonales". Des 3 espèces, *A. subfuscus* est la plus largement répartie dans les régions naturelles. Elle se trouve en grande abondance dans les réserves forestières de Catskill et Adirondack dans l'état de New York, les régions boisées du Nord de la Nouvelle-Angleterre, la Nouvelle Ecosse et certaines parties du Québec.

On a tenté de déterminer le mécanisme probable de l'importation et de la dispersion qui a conduit à la répartition actuelle. Le schéma fondamental est que la plupart des espèces, sinon toutes, ont été introduites à plus d'une occasion. Le moyen d'introduction le plus probable a été le transport avec des plantes importées. Bien qu'il ne soit pas possible d'éliminer la décharge de lest comme source d'introduction des limaces européennes dans certains secteurs côtiers, il apparait que ce n'est pas là le principal mode d'importation. Apparemment les portes de pénétration les plus habituelles ont été les zones urbaines anciennes et étendues. De ces biotopes primaires, les limaces ont été transportées dans des biotopes secondaires, sans doute parmi les végétaux transplantés, et de là elles se sont dispersées à la fois activement et passivement. Beaucoup d'espèces montrent une remarquable capacité de dispersion active dans les habitats qui leur conviennent. Cette capacité avait été minimisée par les précédents auteurs.

RESUMEN

ZOOGEOGRAFIA Y ECOLOGIA DE ARIONIDOS Y LIMACIDOS INTRODUCIDOS EN EL NORESTE DE NORTE AMERICA

L. F. Chichester y L. L. Getz

Se estudió la distribución y ecología de 12 especies. Se visitaron 770 lugares en 8 estados y 5 provincias; de estos, 510 lugares dieron, por los menos, 1 especie importante. Tres especies fueron encontradas en mucha abundancia: *Deroceras reticulatum*, *Arion fasciatus* y *A. fuscus*. Se discute el status de los 3 componentes morfológicos del complejo de *A. fasciatus;* estos se tratan como especies separadas en el estudio. Se registraron nuevas localidades para *A. intermedius*, *A. hortensis*, *A. subfuscus*, and *D. caruanae*.

Las 3 especies más ampliamente distribuidas tienen preferencias ecológicas diferentes. *A. subfuscus* es primeramente una babosa de bosque. *D. reticulatus* es de campo. *A. fasciatus* se distribuye major en áreas naturales - más abundante en situaciones ecotonales - apareciendo en gran cantidad en las reservas forestales de Catskill y Adirondack en el estado de New York, y regiones forestales del norte de Nueva Inglaterra, Nueva Esocia y en partes de Quebec.

Se intentó determinar el mecanismo probable de la introdución de las especies, y la dispersión que condujo a la presente distribución. El patrón básico es de que la mayoría, sino todas, de las especies, fueron introducidas en más de una ocasión. El medio más probable fué con la introducion de plantas. Aunque no se elimina como posible, el acarreo de balastro, como una causa de introdución de babosas europeas en ciertas areas costeras, no parecería que esa fuera la principal. Aparentemente, los puertos de entrada han sido las grandes y bien establecidas areas urbanas. De estos sitios primarios las babosas fueron transportadas a otros secundarios, probablemente con plantas, por los cual la dispersión fue activa y pasiva. Algunas especies muestran considerable facultad para dispersarse activamente en habitats favorables. Esta facultad habia sido descontada por los autores previos.

АБСТРАКТ

ЗООГЕОГРАФИЯ И ЭКОЛОГИЯ СЛИЗНЕЙ АРИОНИД И ЛИМАЦИД, ЗАВЕЗЕННЫХ В СЕВЕРО-ВОСТОЧНУЮ ЧАСТЬ СЕВЕРНОЙ АМЕРИКИ

Л.Ф.ЧИЧЕСТЕР И Л.Л.ГЕТЦ

Изучено географическое и экологическое распространение 12 видов арионид и лимацид, завезенных в северо-восточную часть Северной Америки. Было обследовано 770 пунктов в 8 штатах и 5 провинциях; на 510 из этих участков обнаружен по крайней мере 1 интродуцированный вид, 3 вида (***Deroceras reticulatum***, ***Arion fasciatus*** и ***A. subfuscus***) оказались весьма многочисленными. Обсуждается таксономический статус 3-х морфологических компонентов комплекса ***A. fasciatus***; в исследовании эти компоненты рассматриваются как самостоятельные виды. Обнаружены новые места обитания для ***A. intermedius***, ***A. hortensis***, ***A. subfuscus*** и ***D. caruanae***.

Различаются по своей экологии 3 наиболее широко распространенных вида: ***Arion subfuscus***, который в основном является лесным слизнем; ***Deroceras reticulatum*** - по преимуществу полевой слизень; ***A. fasciatus*** - наиболее обилен в экотонах. Из 3-х видов - ***A. subfuscus*** наиболее широко распространен в природных местообитаниях. Он встречается в массе в Кэтскиллском и Адирондакском лесных заповедниках, в штате Нью-Йорк, в лесных районах северной части Новой Англии, Новой Шотландии и, частично, в Квебеке.

Была сделана попытка определить возможный механизм завоза и расселения, которые привели к современному распространению. Основная схема состоит в том, что большинство видов, если не все, были интродуцированы более одного раза. Наиболее вероятный способ инородукции - с ввозимыми растениями. Хотя было невозможно исключить разгрузку балласта, как источника завоза европейский слизней в некоторых прибрежных областях, видимо это - не главный способ их завоза. По-видимому, наиболее обычными путями завоза слизней были крупные, давно развившиеся городские районы. Из этих первичных участков слизни были перенесены, возможно с растениями, во вторичные участки, из которых они расселились активно и пассивно. Некоторые виды обнаруживают значительную способность активно расселяться в подходящей среде. Эта способность не принималась в расчёт предшествующими авторами.

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STRUCTURE AND FUNCTION OF THE REPRODUCTIVE ORGANS OF THREE SPECIES OF *APLYSIA* (GASTROPODA: OPISTHOBRANCHIA)[1]

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ABSTRACT

The reproductive tracts of 3 species of aplysiid gastropods show incomplete separation of the efferent channels for the male and female gametes. The system functions so as to translocate oöcytes (by ciliary action) during oviposition, to expel autosperms (by ciliary and muscular action), and to receive allosperms transferred during chain-copulation. Complex ciliary tracts and typhlosoles serve to separate these various activities. The functions of the septa and the efferent channels of the common hermaphrodite duct are described. The gradual build-up of the spawn mass was followed in serial sections through the entire nidamental gland complex of specimens killed during various phases of activity. The efferent passage of female gametes during oviposition, and the placement of the penis during copulation, were elucidated. Self-fertilization is prevented by the retention of autosperms in a physiologically immature, non-motile state, so that the efferent oöcyte-stream during oviposition meets immature autosperms, and fertilization is brought about by active allosperms from the receptaculum seminis. Artificial self-fertilization can be induced if semen exchange is permitted before the gametes are mixed. Activation of spermatozoa occurs only after their exchange during copulation; this activation is brought about by the secretions of the female tract of the copulation-recipient, but attempts to isolate the activating agent were not successful.

Previous accounts of spermiogenesis and of the structure of the mature spermatozoon are incorrect in many respects. The nucleus of the aplysiid spermatozoon is a helical structure, and the helically disposed pair of mitochondrial strands travels into the head along with the flagellum, which originates close to the anterior tip of the gamete. The structure of the spermatozoon and of the sperm-storing organs were investigated with the electron microscope. The spermatheca is shown to function solely to digest stray gametes, and it is proposed that it should be termed the gametolytic gland.

INTRODUCTION

The earliest account of the functional morphology of aplysiid reproductive organs was given by Robert (1890), who worked with *Aplysia fasciata* and *A. depilans;* this paper described oviposition as observed in dissections. The monograph of Mazzarelli (1893) introduced a number of minor errors, principally involving the role of the spermatheca, which were corrected in the admirable memoir of Eales (1921). Eales reviewed in detail the early literature and elucidated for the first time the course of the efferent channels through the nidamental gland complex. She also investigated the route followed by allosperms during copulation in *A. punctata*. An unpublished thesis by

[1]This paper is respectfully dedicated to Dr. Nellie B. Eales, D.Sc., who has pioneered work on aplysiids and other molluscs for over forty years, on the occasion of her retirement from the Editorship of *The Proceedings of the Malacological Society of London* in 1969.

Lloyd (1952) dealt mainly with other opisthobranchs, but included a number of original observations on *A. punctata*. Robert (1888), Tuzet (1940) and Franzén (1955) gave conflicting accounts of spermiogenesis in aplysiids; Ries & Gersch (1936) and Bolognari (1960) described features of the structure of the mature ovum of *Aplysia*.

While the basic features of the anatomy of the aplysiid reproductive system are now beyond dispute, a re-investigation of the functional aspects was necessary, for the following reasons: (1) the conflicting claims regarding spermiogenesis needed clarification; (2) the ultrastructure of the spermatozoon promised to yield data of interest in relation to knowledge of the nudibranch spermatozoon (Thompson, 1966); (3) the functioning of the incompletely divided efferent system was of interest because the means by which self-fertilization is prevented was uncertain; (4) the mechanism of physiological activation of the male gametes was unknown - whether activation resulted from the action of secretions of the male efferent tract, or as the consequence of some activity of the copulation-recipient, was not understood; (5) the recent claim by Vicente (1966) that surgical removal of the rhinophoral areas of *Aplysia* resulted in heightened reproductive activity, required verification; (6) the cytological build-up of the various layers and components of the spawn-mass was incompletely understood; (7) the mode of propulsion of the male and female gametes during their efferent translocation required elucidation; (8) and finally, the increasing popularity of *Aplysia* in neurophysiological and other research work raised interest in the possibility of rearing aplysiids to furnish more abundant experimental material - it was thus hoped that it might be possible to carry out artificial fertilizations. The technique of artificial fertilization would enable also the testing of Eales' (1921) theory that the oöcytes are immature as they meet autosperms during their efferent journey in the process of oviposition.

This paper records positive progress in all these lines of enquiry, but it will be seen that a number of further problems have been revealed, which remain to be solved.

The nomenclature of the reproductive organs used here is in the main orthodox, but it has proved necessary to re-name the spermatheca (bursa copulatrix, or vesicle of Swammerdam). It is necessary to call this sac the gametolytic gland, because it does not receive the penis during copulation nor does it store viable allosperms, but merely functions to digest and destroy stray gametes of both sexes. It appears that the anatomical homologue of this sac in the dorid nudibranchs has a rather different role, and for that reason the name bursa copulatrix should be retained (Thompson, 1966).

MATERIAL AND METHODS

Aplysia depilans and *A. fasciata* were collected from shallow water and examined alive at Arcachon, Gironde. Similarly, *A. punctata* was studied alive at Falmouth, Cornwall and at Port Erin, Isle of Man. The taxonomic differences between these 3 species are summarised by Bebbington & Thompson (1968). Material for histology was fixed in Bouin's fluid, sometimes with glutaraldehyde substituted for formaldehyde, in Zenker's fluid with or without acetic acid, in 10% formalin, or in Lewitsky-saline (Baker, 1958). Amyl acetate was employed as a clearing agent following dehydration, and the blocks were cast either in Hance's rubber wax (Gurr) or in ester wax (Steedman, 1960). Sections were stained in Mayer's haemalum with alcian blue 8 GS (Steedman, 1950), in azan or in iron haematoxylin.

Material for the electron microscope was fixed in phosphate-buffered 25% glutaraldehyde with added sucrose, washed in the buffer, and post-fixed in

1% osmic acid in the buffer. Araldite sections were stained with saturated uranyl acetate in 70% alcohol.

ANATOMY AND HISTOLOGY OF THE CONDUCTING SYSTEM

In all 3 species the efferent system is rather similar, and the following account refers to all, except where indicated.

Collecting tubules from the ovotestis (Plates 1,A; 2,B; 4,A) unite (Fig. 6) and lead via an initial narrow ciliated tube (non-ciliated, according to Eales, 1921), into the dilated vesicula seminalis (Figs. 1, 6, 10; Pl. 4,C), in which morphologically mature autosperms are stored. In transverse sections it can be seen that approximately ⅓ - ½ (in the different species) of the endothelium of the vesicula seminalis is ciliated, while the remainder is non-ciliated (Figs. 4,D; 7,A). The living organ appears cream-white in colour, due to the masses of autosperms within it. It is enclosed in a thin layer of mixed circular and longitudinal smooth muscle bundles (Fig. 4,D). Towards its distal extremity the vesicula seminalis again narrows (Fig. 1) and a minute longitudinal typhlosole makes its appearance (Figs. 4,E; 7,C). This typhlosole bears especially strong cilia and subtends the internal autospermal groove. In transverse sections of this second narrow region of the hermaphrodite duct, it can be seen that all the endothelial cells are ciliated.

The hermaphrodite duct is then attached by a delicate membrane to the surface of the nidamental gland-complex (mucous gland and albumen gland). It forms a broad loop (Fig. 2,B) before disappearing below the surface of the gland. The divergence of the male (autospermal) and female (oviducal) channels was traced in serial sections of the whole nidamental gland region.

The male channel continues distally as the internal autospermal groove on the major typhlosole (Figs. 2,B; 4,F; 9,A; 10,C; Pl. 1,B) of the succeeding wide distal hermaphrodite duct. The groove could be traced past the junction of the duct with the stalk of the gametolytic gland, until it leads into the external autospermal groove (Fig. 5,H) running down the right antero-lateral body wall to the penis (Fig. 5,G). At the junction with the gametolytic gland (Fig. 3,B), the groove receives the secretion of the prostate gland. In *Aplysia punctata* and *A. fasciata* this gland is well developed and forms a lump (Fig. 3,A) which may be discerned in gross dissections. The lobules of this prostate are lined with heavily laden secretory cells. In *A. depilans*, however, the stalk gland is very small; it can only be detected by sectioning the whole area. Correlated with this, the internal autospermal groove is strongly glandular in *A. depilans* (Fig. 9,A; Pl. 1,B), and the prostatic secretions are accordingly poured upon the autosperms all along the course of their passage. Eales (1921) suggested that the stalk gland secretion serves to agglutinate stray gametes entering the gametolytic gland, but she gave no evidence for this. The external autospermal groove (Fig. 5,H) in all the species is strongly ciliated (the cilia are, in fixed preparations, 7 μm in height, compared with 4μm over the rest of the lateral epithelium), and leads to the base of the penis. This intromittent organ is normally held in a retracted state inside its sheath. When erect, the autospermal groove can be seen to travel along the side of the penis (Fig. 5,G) to its tip. The form of the penis varies in the 3 species. In *A. punctata*, it is a broad, spatulate, darkly pigmented structure. In *A. fasciata* it is greatly elongated and pale in colour. In *A. depilans* the darkly pigmented, somewhat stout penis bears at its base a number of spiny warts (Fig. 10) the function of which will be discussed later. Pruvot-Fol (1960, fig. 9) illustrates the penis of *A. depilans* wrongly captioned *A. fasciata*. Illustrations showing dissections of the penial

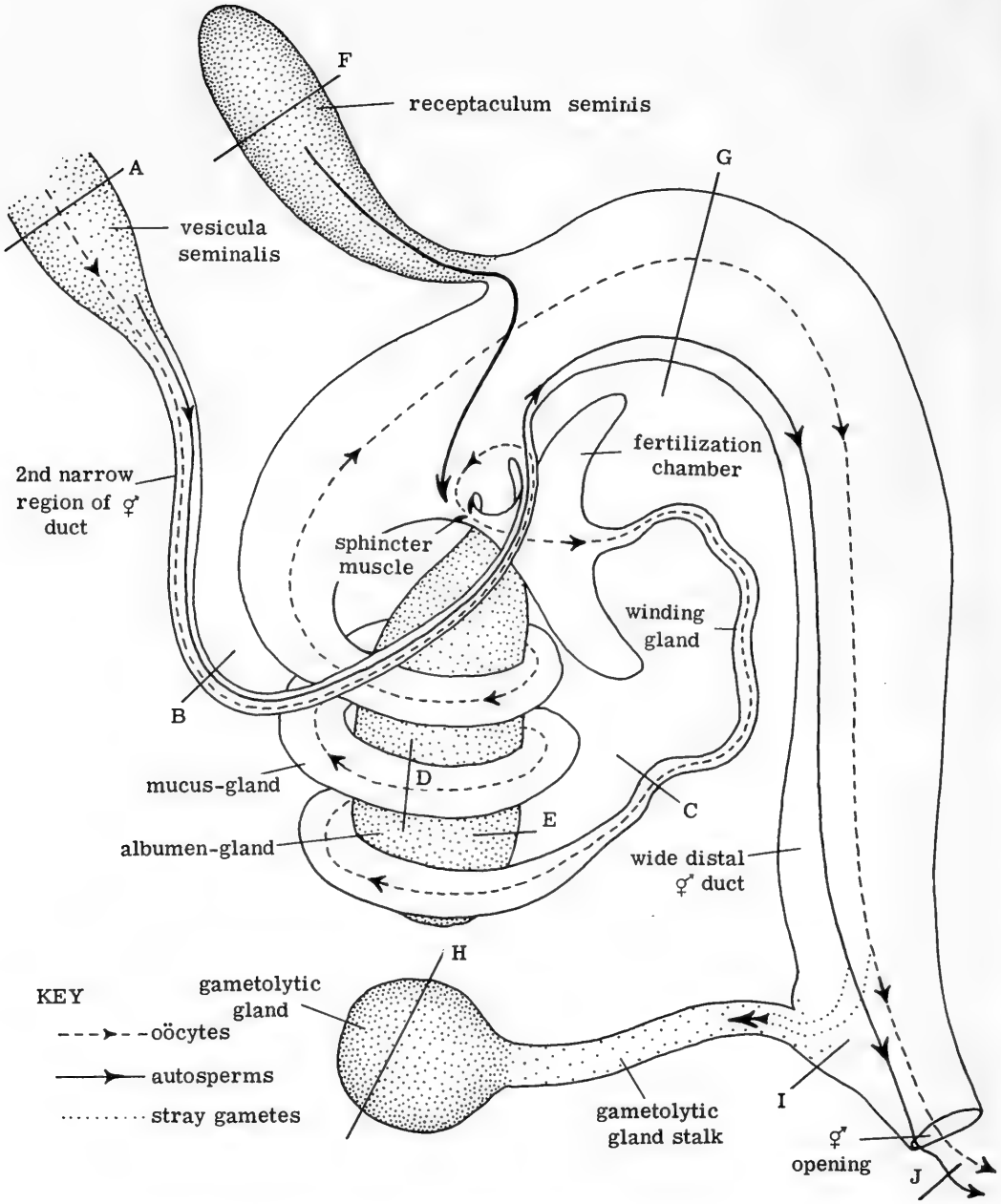


FIG. 1. Diagrammatic representation of the aplysiid reproductive system. Arrows show the courses of the outgoing gametes during copulation and oviposition (which may occur simultaneously). A section through region A is illustrated in Figs. 4,D; 7,A; Pls. 2,A; 4,B, C and D; through region B in Figs. 4,E; 7,C; Pl. 1,F; through region C in Fig. 7,D; Pl. 2,C; through region D in Figs. 5,C,D; 8,A,B; Pl. 2,D; through region E in Figs. 5,E; 7,E; through region F in Fig. 5,A; Pls. 1,D; 2,E; 4,E, F; through region G in Figs. 4,F; 9,A; through region H in Figs. 5,F; 9,D; Pl. 2,F; through region I in Fig. 5,I; through region J in Fig. 5,H.

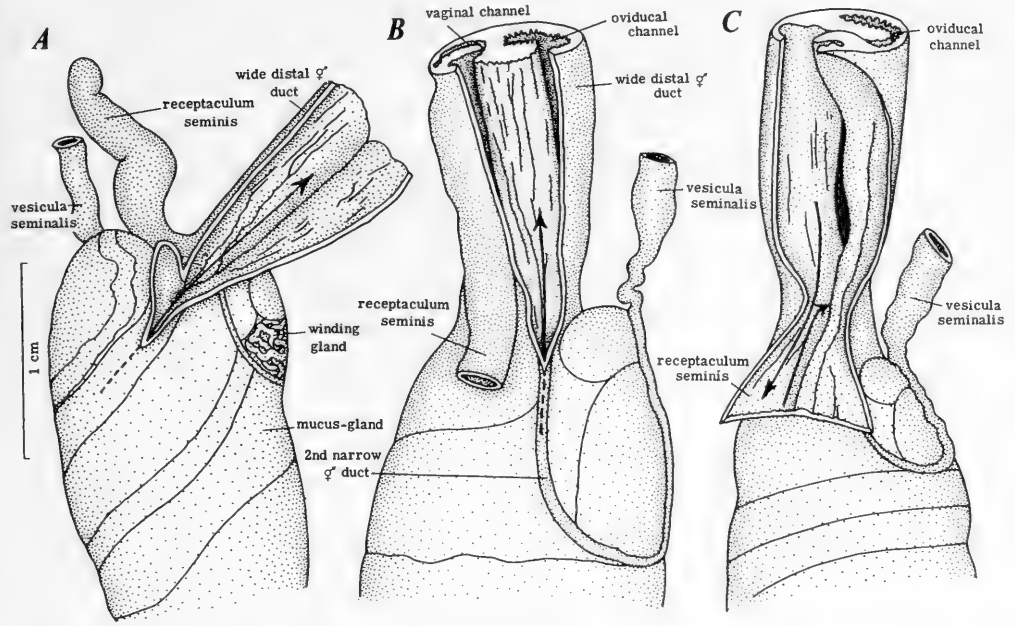


FIG. 2. Dissections to show the proximal region of the wide distal hermaphrodite duct of *Aplysia fasciata*. A, The female efferent channel from the mucous gland. B, The autospermal efferent channel from the vesicula seminalis and 2nd narrow hermaphrodite duct. C, The vaginal channel for incoming allosperms, leading into the receptaculum seminis. The arrows show the course of allosperms during reception at copulation, and during their emigration during oviposition.

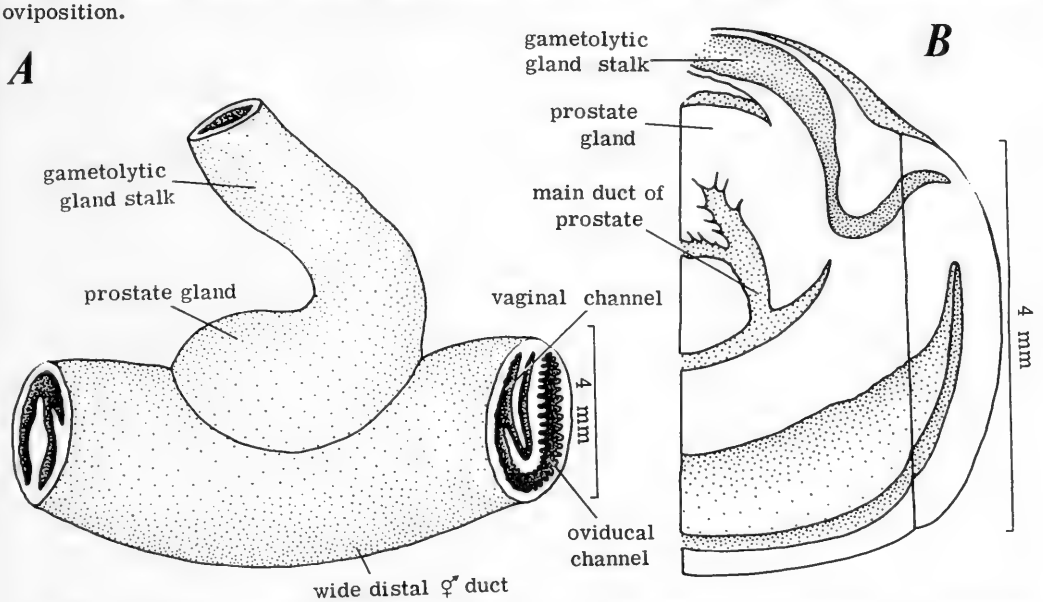


FIG. 3. The stalk of the gametolytic gland of *Aplysia fasciata*. A, Dissection to show the internal divisions of the wide distal hermaphrodite duct proximal to and distal to the junction with the gametolytic gland stalk. B, Diagram to show the points of entry of the prostate gland and the gametolytic gland stalk.

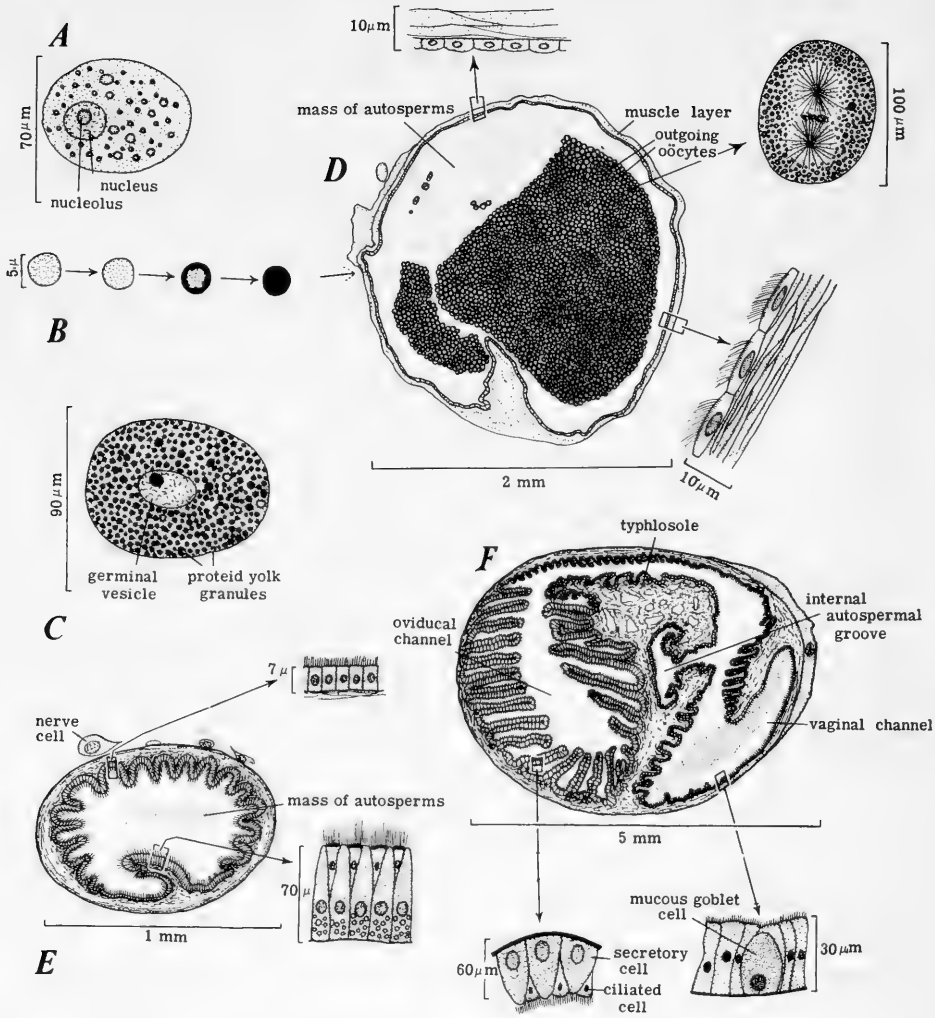


FIG. 4. Sections through reproductive organs of *Aplysia fasciata;* Bouin and azan. A, Young oöcyte from the ovotestis, showing the commencement of condensation of yolk reserves, which at this stage stain blue with azan; the nucleolus stains similarly. B, Stages in the conversion of a yolk granule into the definitive form which stains red with azan. C, Mature oöcyte, before ovulation, with nucleolus and yolk granules staining red with azan. D, Vesicula seminalis during oviposition, showing the histological differentiation of the endothelium into ciliated and non-ciliated regions (limits indicated by arrows with dashed heads). Insets show the structure of the 2 endothelial types, and an oöcyte without nuclear membrane and with the first reduction division arrested during metaphase. The tubular organ contains masses of oöcytes following their efferent route, passing through the inert autosperms. E, More distal region of the vesicula seminalis, showing the endothelium to be wholly ciliated and folded to form the internal autospermal groove. Insets show portions of the endothelium, at a greater magnification. F, Wide distal hermaphrodite duct, showing the division of the lumen into 3 channels. In this species the internal autospermal groove is non-glandular. Insets show the endothelium in different parts of the duct, at a greater magnification.

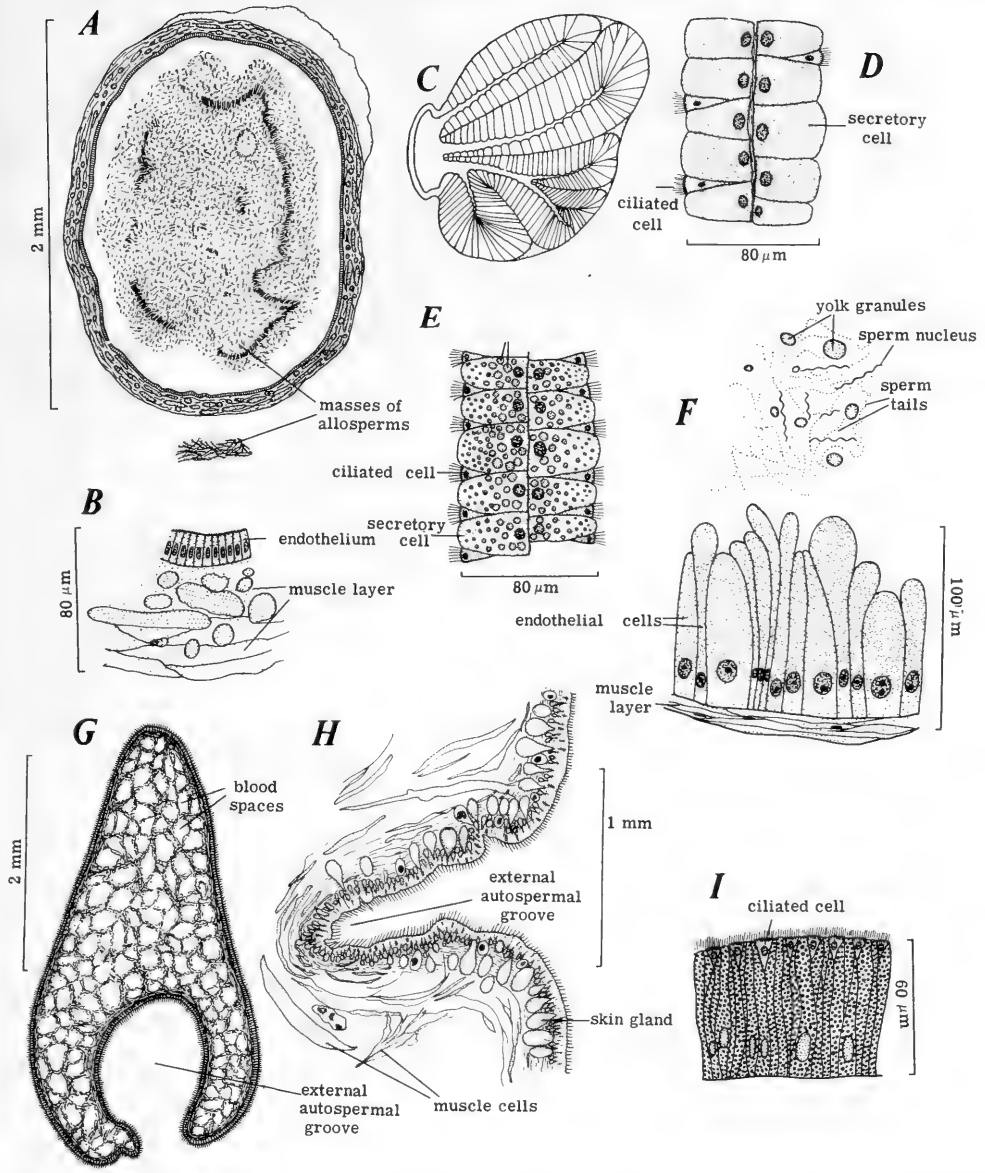


FIG. 5. Sections through reproductive organs of *Aplysia fasciata;* Bouin and azan. A, Receptaculum seminis, showing the contained masses of allosperms; the gap between the allosperms and the endothelium is an artefact. B, Portion of the same, at a greater magnification. C, Mucous gland, showing the great increase in secretory area attained by folding of the endothelium. D, Portion of the same, at a greater magnification. E, Albumen gland, showing the endothelial cells loaded with amber secretory droplets. F, Gametolytic gland, showing the secretory endothelium and male and female gametes in various stages of degradation. G, Penis, near the tip, showing the external autospermal groove, and the central area, rich in blood-spaces. H, Antero-lateral body wall, showing the external autospermal groove. I, Prostate gland endothelium.

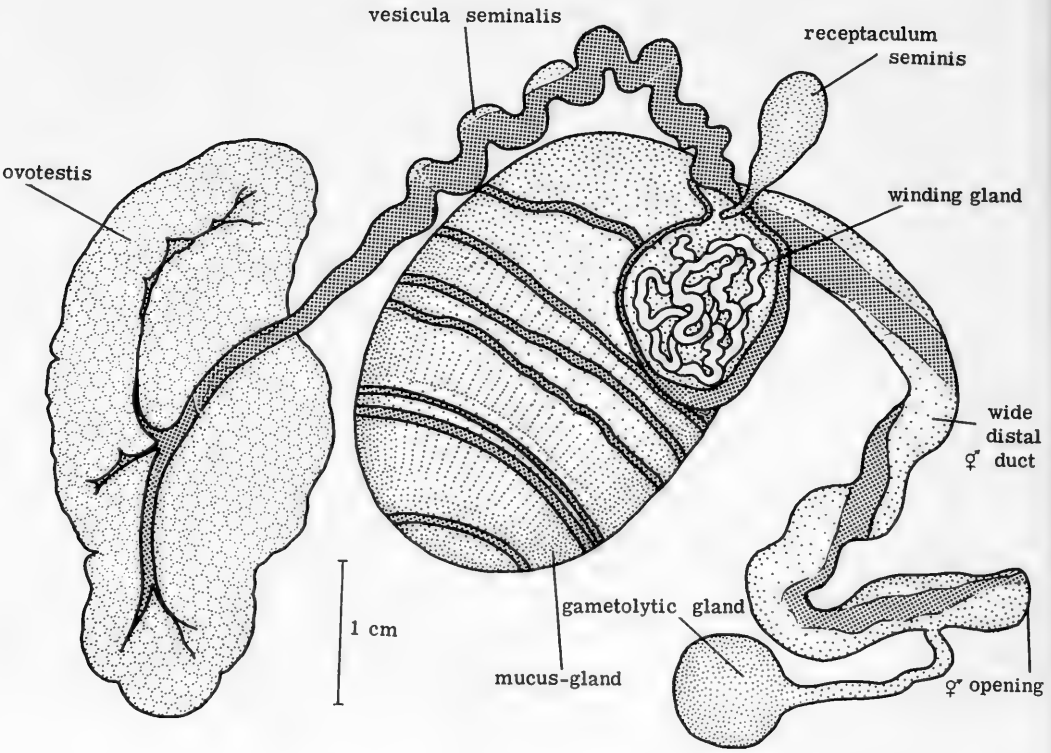


FIG. 6. Dissection to display the reproductive system of *Aplysia fasciata* during oviposition. The oöcyte-stream (stippled) is visible through the walls of the efferent ducts.

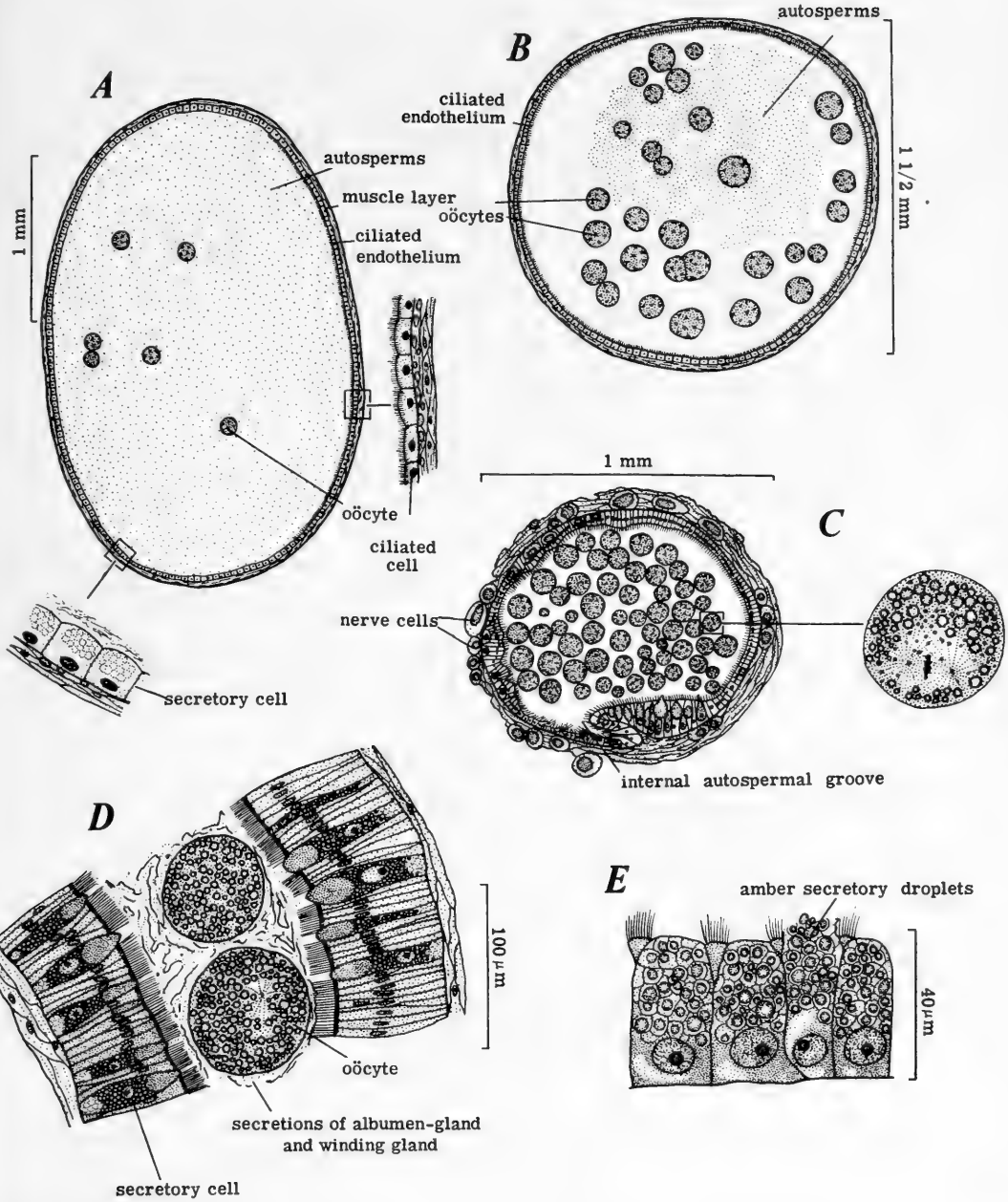
FIG. 7. Sections through reproductive organs of *Aplysia depilans* during oviposition; Bouin and azan.

A, Middle region of the vesicula seminalis (ligatured before fixation), to show the histological differentiation of the endothelium into ciliated and non-ciliated regions (limits indicated by arrows). Insets show the structure of the 2 endothelial types.

B, More distal region of the vesicula seminalis, showing the wholly ciliated endothelium.

C, 2nd narrow region of the hermaphrodite duct, showing the wholly ciliated endothelium and the internal autospermal groove. Inset is an oöcyte showing the nucleus arrested at the metaphase of the first reduction division.

D, Winding gland, showing the oöcyte-stream receiving the secretion of the endothelium of this region of the efferent female duct. Amber secretions from the albumen gland are also evident in the lumen.



Legend to Fig. 7 (cont.).

E, Secretory endothelium of the albumen gland, showing the phase of active secretion of amber spherules into the lumen. Oöcytes do not enter the gland during their passage to the mucous gland.

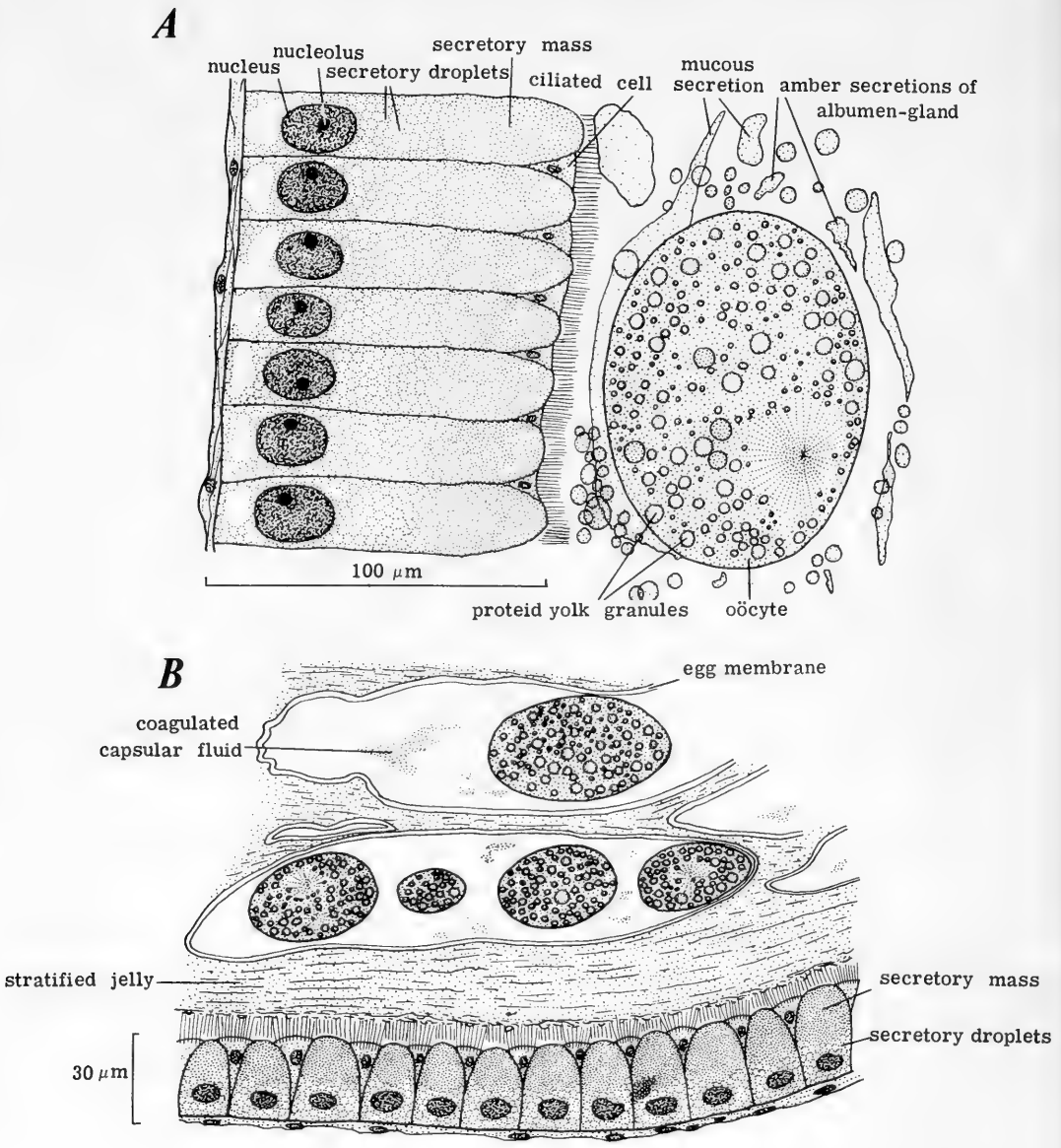


FIG. 8. Sections through reproductive organs of *Aplysia depilans* during oviposition; Bouin and azan.

A, Early part of the mucous gland, showing the addition to the stream of ova of mucus from the endothelial secretory cells.

B, Later part of the mucous gland, showing the stratification of the mucus around the stream of ova. Egg capsules are now evident.

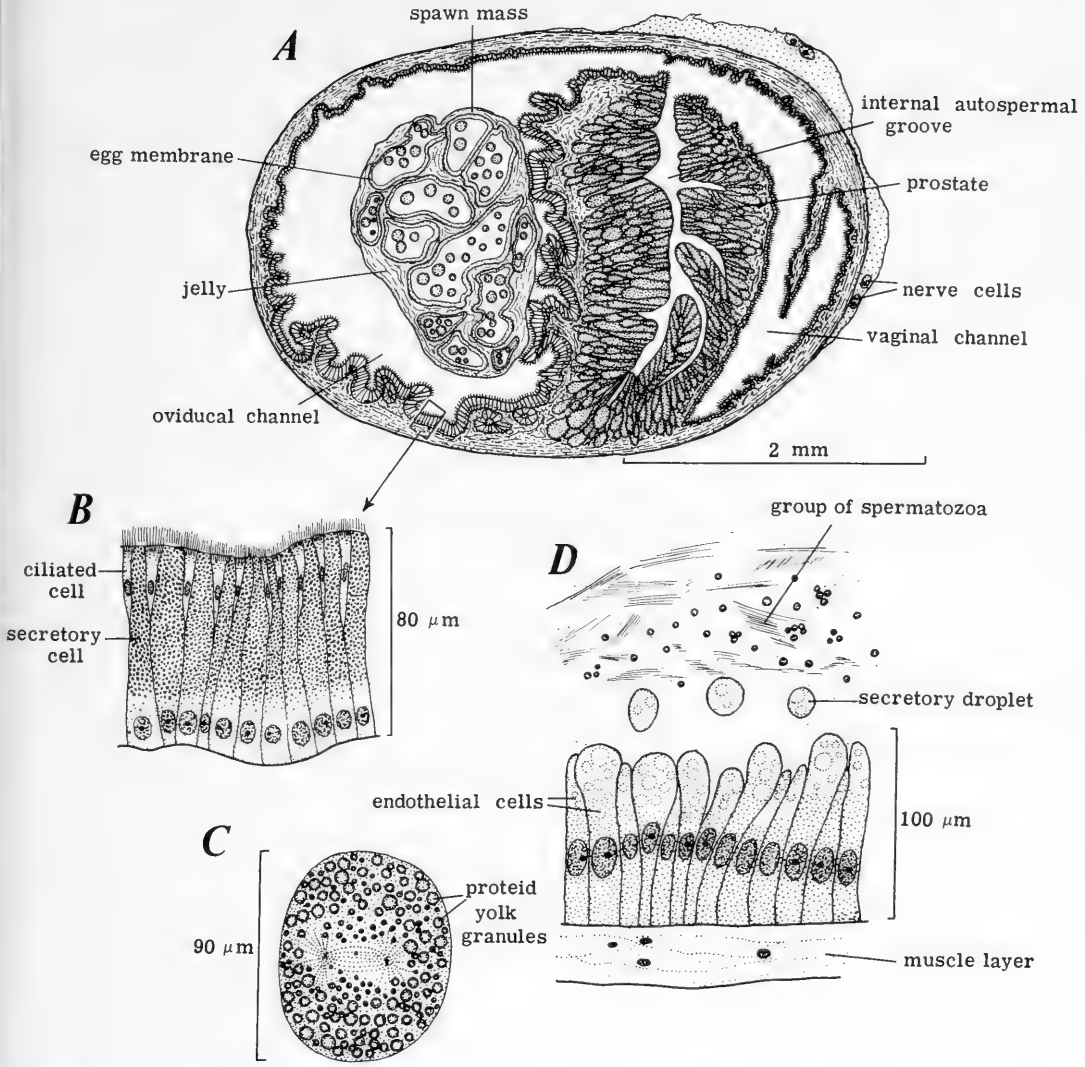


FIG. 9. Sections through reproductive organs of *Aplysia depilans* during oviposition; Bouin and azan.

A, Wide distal hermaphrodite duct just beyond the mucous gland, showing the fully formed egg-string passing down the female oviducal channel. The section also shows the autospermal channel with its prostatic endothelium, and the vaginal channel.

B, Secretory endothelium of the female channel of the wide distal hermaphrodite duct.

C, Ovum from the egg-stream shown in A, showing the continuing arrest of the nuclear changes at the metaphase of the first reduction division.

D, Secretory endothelium of the gametolytic gland, showing portions of eggs and spermatozoa in various stages of degradation.

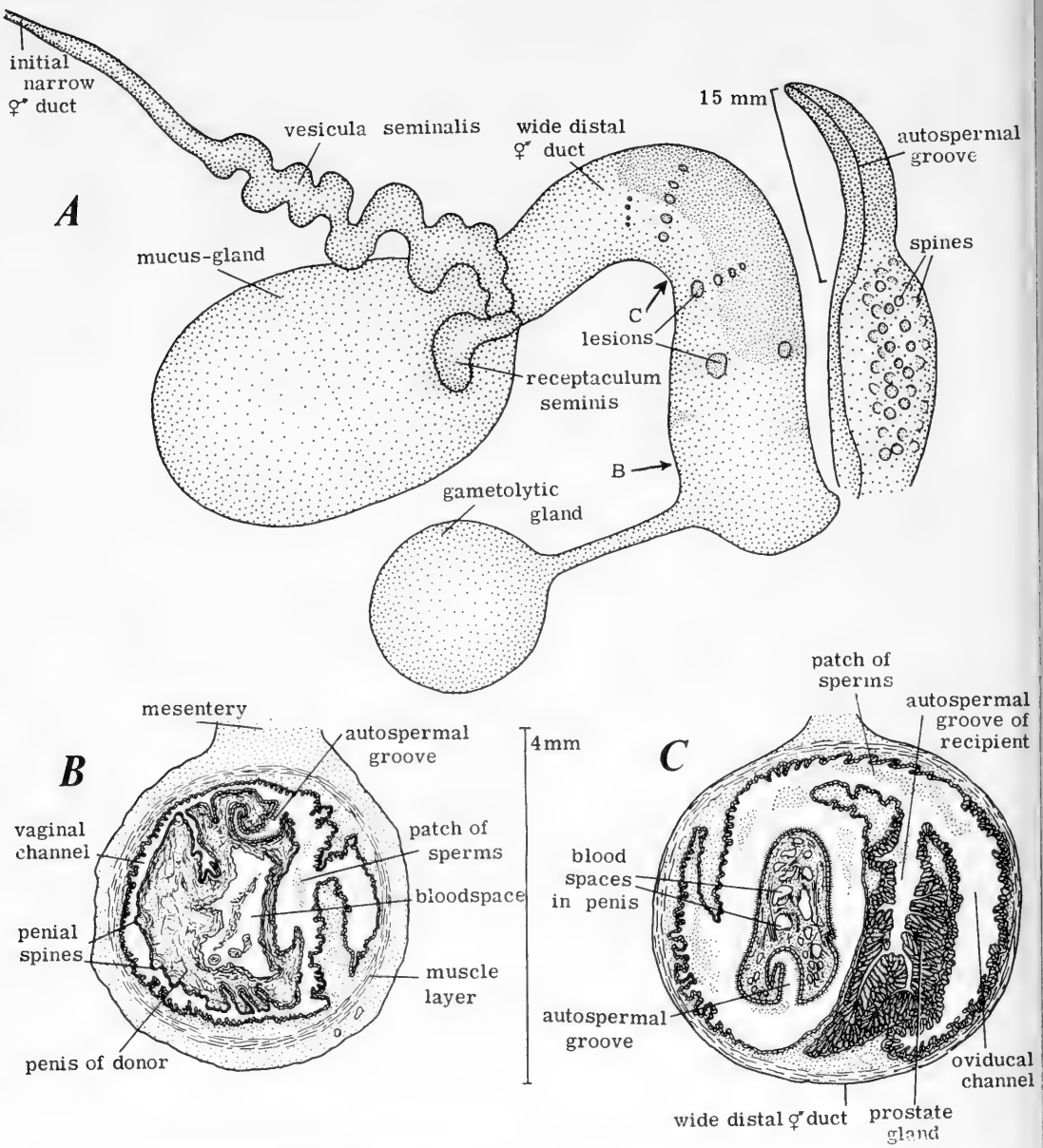


FIG. 10. *Aplysia depilans* during copulation.

A, Dissection of the reproductive system of a recipient specimen with the donor penis *in situ* and showing perforations in the wall of the wide distal hermaphrodite duct caused by the penial spines of the donor. A drawing of the penis is placed alongside.

B,C, Sections through the wide distal hermaphrodite duct at the levels indicated, showing the penis with its autospermal groove in the vaginal channel; hot Bouin and azan.

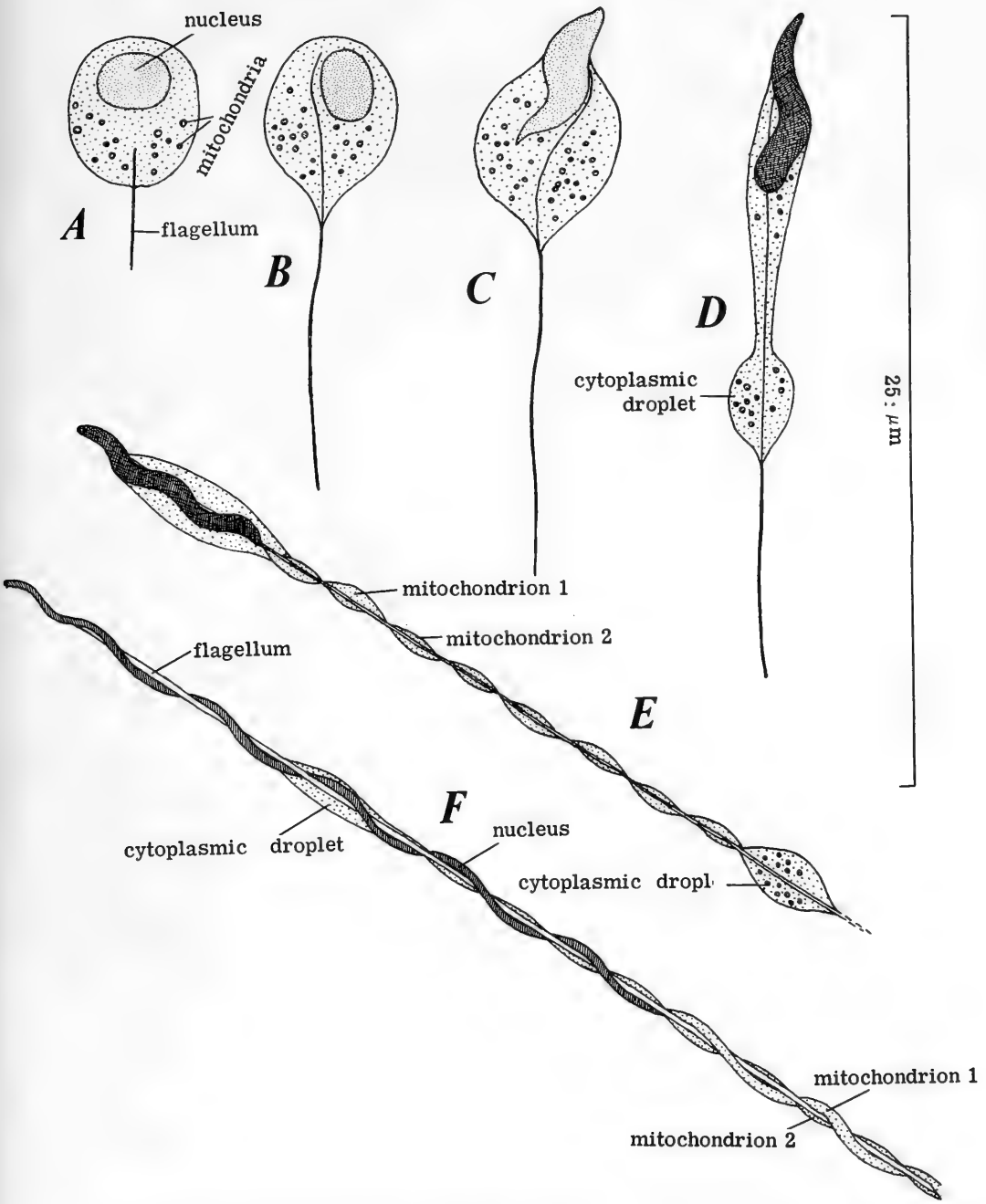


FIG. 11. Stages in spermiogenesis of *Aplysia punctata*. Camera lucida drawings from life, in saline after staining with intravital dyes. A, Early spermatid. B, Spermatid with elongating flagellum. C, Spermatid showing the beginning of the nuclear helix. D, Spermatid with elongating nucleus and formation of cytoplasmic droplets. E, Spermatid with dual mitochondrial helices. F, Spermatozoon with elongated nuclear helix, flagellum reaching almost to the anterior extremity, and dual mitochondrial helices.

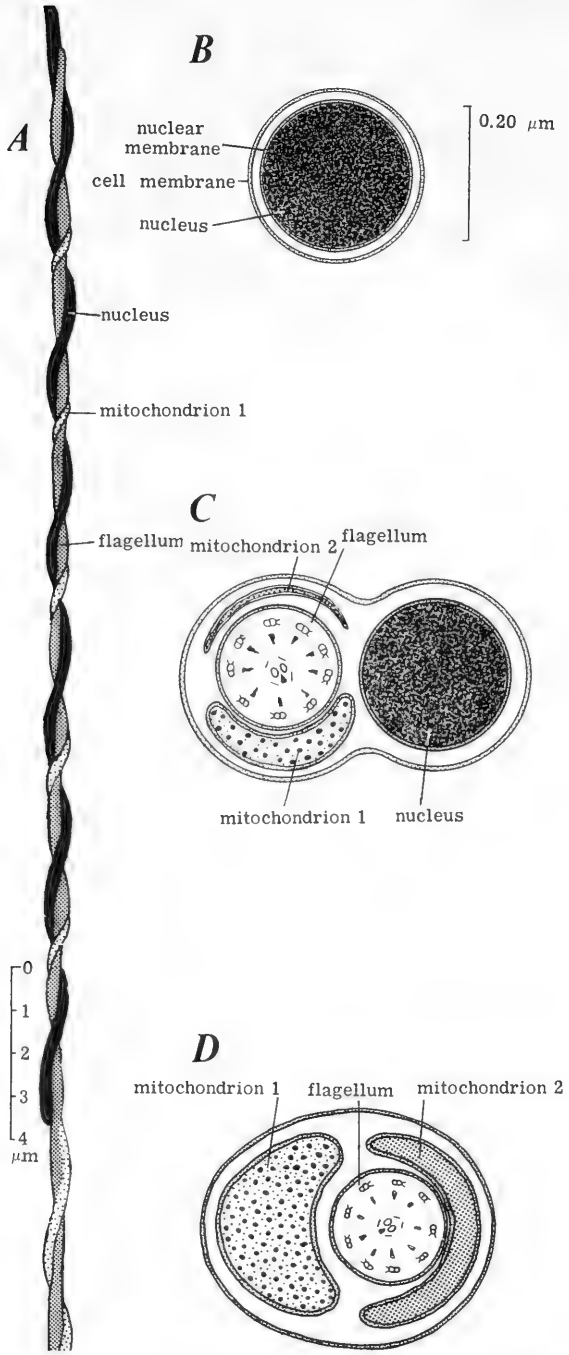


FIG. 12. The aplysiid spermatozoon; based upon electron micrographs. A, Anterior end of a spermatozoon. B, Section through the head-tip. C, Section through the mid-region of the head. D, Section through the principal-piece.

apparatus of all 3 species are given by Bebbington & Thompson (1968).

From the point where the male and female channels diverge, the female passage proceeds to a narrow tubule (Fig. 1), receiving en route the duct from the albumen gland. This gland is large and lies centrally in the nidamental gland complex. In *Aplysia punctata* the gland is visible after gross dissection, but in the other 2 species the mucous gland must be cut away to reveal it. The secretory cells of the albumen gland (Figs. 5,E; 7,E) contain numerous secretory droplets which comprise masses of amber granules. Wedge-shaped ciliated cells alternate with the secretory cells. The female channel narrows at a sphincter muscle before dilating to form a capacious fertilization pouch (Fig. 1). From this pouch a convoluted tube, the winding gland, emerges (Figs. 1; 6; 7,D). This is lined by columnar ciliated endothelial cells (Pl. 2,C), containing large numbers of small secretory droplets that stain blue with azan and with alcian blue. This winding gland then dilates and proceeds along a complex course, forming the mucous gland region of the female duct. The windings of the mucous gland (Figs. 1; 2; 5,C,D; 6; 8; Pl. 2,D) lie superficially over the albumen gland. The mucous gland tubules are much folded (Fig. 5,C) to increase the area for secretion. The secretory cells, interspersed with wedge-shaped ciliated cells, contain secretory mucous droplets which take up alcian blue strongly. From the mucous gland the oviducal channel once again joins the autospermal channel in the wide distal hermaphrodite duct (Fig. 2, A).

The wide distal hermaphrodite duct (Figs. 1; 2; 6) is stout and sections show that it is divided longitudinally by incomplete internal septa (Figs. 4,F; 9,A). In particular, 2 typhlosoles are conspicuous, and these function to separate the gametes. The major typhlosole, bearing the internal autospermal groove (Figs. 4,F; 9,A), effectively partitions the duct so that the female gametes during oviposition are held in approximately one half of the duct. This female channel is lined by secretory cells, interspersed with wedge-shaped ciliated cells (Figs. 4,F; 9,B). This secretory endothelium persists only as far as the level of the gametolytic gland stalk; from hence distally the female channel is non-glandular.

The remaining moiety of the lumen of the wide distal hermaphrodite duct serves for the conduction of the penis of an allosperm-donor during copulation. This vaginal channel (Figs. 2,C; 3,A; 4,F; 9,A; 10,B and C) leads up into the receptaculum seminis (Figs. 1; 2; 6, 10) which lies close to the nidamental gland complex, to which it is united by a delicate membrane. The receptaculum is a blind diverticulum of the hermaphrodite duct and is invested with a strong muscular coat (Figs. 5,A and B). The endothelial cells are histologically simple and bear a clearly visible brush border but are not ciliated. Allosperms received during copulation are stored in this organ (Fig. 5,A; Pls. 1,D; 2,E; 4,E and F; 6; 7).

The gametolytic gland (Figs. 1; 5,F; 6; 9,D; 10; Pl. 2,F) is the site of digestive degradation of stray gametes, and sections show spermatozoa and ova in various stages of demolition. Faint secretory droplets can be distinguished in sections through the endothelial cells and the organ has a strong muscle-coat.

THE OVOTESTIS

The lobules of the ovotestis present a similar appearance in all the species, with male and female gametes developing in close proximity (Pls. 1,A; 2,B; 4,A). The whole organ is invested by delicate muscle fibres. Spermatozoa from the ovotestis are frequently mature in appearance, but are inactive and incapable of fertilization. As will be demonstrated later, sperm-activation and the attainment of fertilizing power occur only after spermatozoa are exchanged at copulation. It is this phenome-

non which prevents self-fertilization, not the immaturity of the female gametes postulated by Eales (1921).

(a) Oögenesis

Oöcyte growth occurs until a diameter of approximately 70μm is reached, when yolk granules first become evident (Fig. 4,A). The first yolk granules stain blue with azan and have a faintly reticulate structure. These reticulations then disappear and the granules are transformed into the definitive reserves, 2-10μm in diameter. This change affects the peripheral area of each granule before it spreads centripetally into the medulla of the granule (Fig. 4,B), when the granule has a hyaline appearance and stains deeply red with azan. Yolk granules in all parts of the oöcyte develop at the same rate; there is no recognizable yolk-nucleus. With the growth and development of the yolk granules in the cytoplasm of the oöcyte, the cell grows to its maximal diameter of approximately 90μm, and the conspicuous single nucleolus changes in staining reaction with azan from blue (4μm in diameter) to red (10μm in diameter), finally disappearing as the oöcyte reaches maturity (Fig. 4,C). The bloated germinal vesicle (Pl. 3,A) may be up to 25μm in diameter, with no visible chromosomes. Each early oöcyte has several nurse-cells around it; these disappear before ovulation.

(b) Spermiogenesis

Spermatids and spermatozoa from the ovotestis of *Aplysia punctata* were examined with bright-field and phase-contrast microscopy, in sea water with the addition of janus green, methylene blue or neutral red. The following account of spermiogenesis is based on various stages found in several different adult specimens; the cytological transformations involved could not be traced in any single spermatid.

From the early spherical spermatid with swollen nucleus and scattered spherical mitochondria, a flagellum begins to emerge from what will become the rear extremity (Fig. 11,A). Internally, the flagellum comes to extend through the greater part of the length of the elongating cell (Fig. 11,B). The nucleus then begins to assume a helical form (Fig. 11,C). The bulk of the cytoplasm migrates rearwards (Fig. 11, D), carrying with it the mitochondria and other finely granular inclusions. As this migration continues, the cytoplasm splits into two globules, one remaining associated with the head of the sperm, the other retreating along the flagellum. As the latter globule travels back, it pays out a pair of elongated mitochondrial strands, spiralling around the flagellum (Fig. 11,E). From the first, these 2 strands are disparate in size; this disparity increases as spermiogenesis continues.

The nucleus continues to elongate, forming more turns of the helix and extending rearwards alongside that part of the flagellum which had already been ensheathed by the double mitochondrial helix (Fig. 11,E). The anterior cytoplasmic globule soon disappears; the posterior globule continues to retreat, paying out the double mitochondrial helix until it finally passes off the rear tip of the spermatozoon (Pl. 3,D). In the last stages of spermiogenesis, it can be seen (Fig. 11,F) that the spiral of the nucleus involves 5-6 full turns, while the number of turns undergone by the mitochondrial spirals behind the level of the head is 28-30. These helices are all clockwise when seen from the front. In the morphologically mature spermatozoon, the break between the helix of the nucleus and that of the larger mitochondrial strand can be distinguished only by staining. The smaller mitochondrial strand cannot then be distinguished with the light microscope, although it could be seen clearly in earlier stages (Fig. 11,F).

During the early stages of spermiogenesis, the developing spermatozoa are embedded head-first in nurse-cells (Pl. 3,B), from which they are freed when

they reach morphological maturity. It is noteworthy that the helical configuration of the nucleus persists after routine histological fixation (Pls. 2,F; 4,F), but that of the principal-piece usually disappears after death.

Observations of spermiogenesis in *Aplysia depilans* and *A. fasciata* indicate that spermiogenesis follows the same course in all the spermatids of the 3 species, contrary to Robert's (1888) claim that a dual method of male gamete formation can be traced in some species of *Aplysia*.

MORPHOLOGICALLY MATURE SPERMATOZOA (Fig. 12)

The spermatozoa of the 3 species of *Aplysia* were investigated with the light and electron microscopes. Differences in the ultrastructure and in the mode of storage and nourishment of the gametes were detected. Some of the data are summarized in Table 1.

Some aspects of the aplysiid spermatozoon have been described in the preceding section dealing with spermiogenesis; studies with the electron microscope have clarified certain structures. Much of the information obtained from these studies is summarized in Fig. 12.

Tuzet (1940) failed to recognize the helical structure of the nucleus, claimed the existence of a discrete mitochondrial middle-piece, and missed the mitochondrial spiral keels of the principal-piece. What she refers to as a filament around the nucleus is in reality the nucleus itself. Franzén (1955) correctly described the spiral of the larger mitochondrial strand, but missed the nuclear helix; like Tuzet, he interpreted the spiral structure of the head as a simple extension of the spiral keel of the principal-piece.

The nucleus of the aplysiid spermatozoon is a cylinder, 0.2 - 0.4μm in diameter, which forms a helix of 5-7 turns (Fig. 12; Pls. 3,C; 5). In sections it appears to consist of coarsely striated material (Pl. 8,C), the majority of the striae running longitudinally, bounded externally by a nuclear membrane. The nucleus extends to the anterior tip of the head; no acrosome could be detected, contrary to Tuzet's (1940) claim. The flagellum originates anteriorly just behind the extremity of the head (Pl. 7,C and D), and thus extends over nearly the whole length of the cell. In sections through the flagellum (Pl. 8,A-C) the familiar 9 peripheral fibre-doublets can be recognized, with arms of the kind first noted in the echinoid flagellum by Afzelius (1959). Radial material extends from each fibre-doublet towards the central pair of fibrils, around which is a set of struts of unknown function. The diameter of the flagellum, surrounded by a delicate membrane, in which microtubules were sometimes discernible, was approximately 0.22μm. The smaller of the 2 mitochondrial strands which spiral around the flagellum contains finely granular material (Pl. 8,A and B); it is not detectable in live material. The larger mitochondrial strand (Fig. 12) is clearly visible in live spermatozoa and forms a projecting spiral keel. This keel in gametes of the nudibranch *Archidoris* (Thompson, 1966) is believed to function in life to convert the uniplanar waves of flagellation into a helical path of progression, by its differential resistance to torque. There is every reason to believe that it has the same role in *Aplysia*. Its contents are coarsely granular in sections (Pl. 8,D-F). Both mitochondria are bounded by discrete membranes consisting of microtubules. The whole gamete is bounded by a strong cell membrane.

The spermatozoon of *Aplysia* is unlike that of any other mollusc so far described in the following respects: (a) the presence of 2 mitochondrial strands, of disparate sizes, forming alternating helices extending over the majority of the length of the cell; (b) the helical disposition of the nucleus; (c) the extension of the flagellum into the centre

TABLE 1. Characteristics of the spermatozoa of 3 species of *Aplysia*

Character	Species		
	A. depilans	*A. fasciata*	*A. punctata*
Length overall	155–158μm	182–185μm	215–228μm
Length of nucleus	25μm	20μm	20μm
Number of spirals visible along whole length of sperm	40–45	40–45	34
Wave-length of spirals	ca. 4μm	ca. 4μm	ca. 6μm
Orientation in the vesicula seminalis	random	random	large groups radially orientated
Orientation in the receptaculum seminis	large groups radially orientated	orientated in swirling groups, not radially	large groups radially orientated
Motility in the ovotestis and vesicula seminalis	non-motile	non-motile	non-motile
Motility in the receptaculum seminis	vigorously motile if released	vigorously motile if released	vigorously motile if released

TABLE 2. Spawn of three species of *Aplysia*

Character	Species		
	A. depilans	*A. fasciata*	*A. punctata*
No. capsules per cm. of spawn (average)	160	118	532
No. ova per capsule (average)	25	43	4
Total eggs per mass (estimate)	3,308,000	25,877,400	135,180
Embryonic period	14–16 days at 25°C	14–16 days at 25°C	20–22 days at 15°C

of the nuclear helix so that it reaches almost to the front tip of the gamete. It may be noted that the aplysiid sperm shows many resemblances to that of the hymenopteran *Dahlbominus* (Wilkes & Lee, 1965); no functional explanation of this remarkable convergence can at present be advanced.

Active aplysiid spermatozoa progress in saline by approximately uniplanar sinusoidal waves of flagellation which are initiated anteriorly and propagated rearwards. Progression is helical, with considerable yawing at low speeds, a more linear path being pursued by fully active individuals.

STORAGE OF SPERMATOZOA *IN VIVO*

In the vesicula seminalis the majority of the spermatozoa are non-motile, whereas gametes artificially liberated from the receptaculum seminis move actively for 2 hrs. or more at 20°C. The former may be termed autosperms, the latter allosperms (Thompson, 1966). In most gastropods autosperms are non-orientated, whereas the allosperms are usually radially arranged with their heads facing, but probably never embedded in, the endothelial lining of the storage organ. In *Aplysia*, the situation is more complex (Table 1). *Aplysia depilans* exhibits the typical situation (Pl. 1,D; Fig. 7,A and B), but in *A. punctata* large numbers of autosperms are radially orientated within the vesicula seminalis (Pl. 4,C and D), while in *A. fasciata* allosperms in the receptaculum seminis (Pl. 2,E) are arranged in swirling groups (each group consisting of thousands of gametes) which may not touch the endothelium. Nourishment of autosperms *in vivo* presumably presents few problems, because their needs are low in the absence of any stimulus to motility. Allosperms, on the contrary, are motile even in the crowded conditions of the receptaculum seminis, and they clearly need more effective means of food-provision. In the receptaculum of *A. depilans* (Pl. 1,D) and *A. punctata* (Pl. 4,E), these needs are met by the radial orientation of the allosperms so that each individual gamete is in contact with the endothelium. In *A. fasciata*, however, the allosperms do not individually make contact with the microvillous endothelial cells of the receptaculum (Pl. 2,E), and in this species a unique system of elongated, swollen microvilli (Pl. 6,A) is present which probably plays a role in nutrient-transmission. Sections examined with the electron microscope show these dilated microvilli, around which groups of allosperms are arranged (Pl. 6,A). In all 3 species sections show that more delicate microvilli (Pl. 8,F) from the receptacular endothelial cells intimately invest many of the allosperms.

DISPOSAL OF WASTE GAMETES

Sections through the gametolytic gland (Figs. 5,F; 9,D; Pl. 2,F) frequently show spermatozoa in various stages of degradation as the result, presumably, of the action of the endothelial secretion. Nuclei are digested before flagella. Semi-digested yolk granules are also often found. The gland therefore functions to remove gametes which have strayed during copulation or oviposition.

ACTIVATION OF SPERMATOZOA

As mentioned earlier, autosperms are in general non-motile, whereas allosperms from the receptaculum seminis exhibit vigorous motility. No ultrastructural difference could be detected between autosperms and allosperms. Some experiments were carried out to discover if activation occurred in the male tract of a copulation donor or in the female tract of a copulation recipient. It was hoped that it might be possible to extract an activating agent from preparations of various parts of the reproductive system. This hope

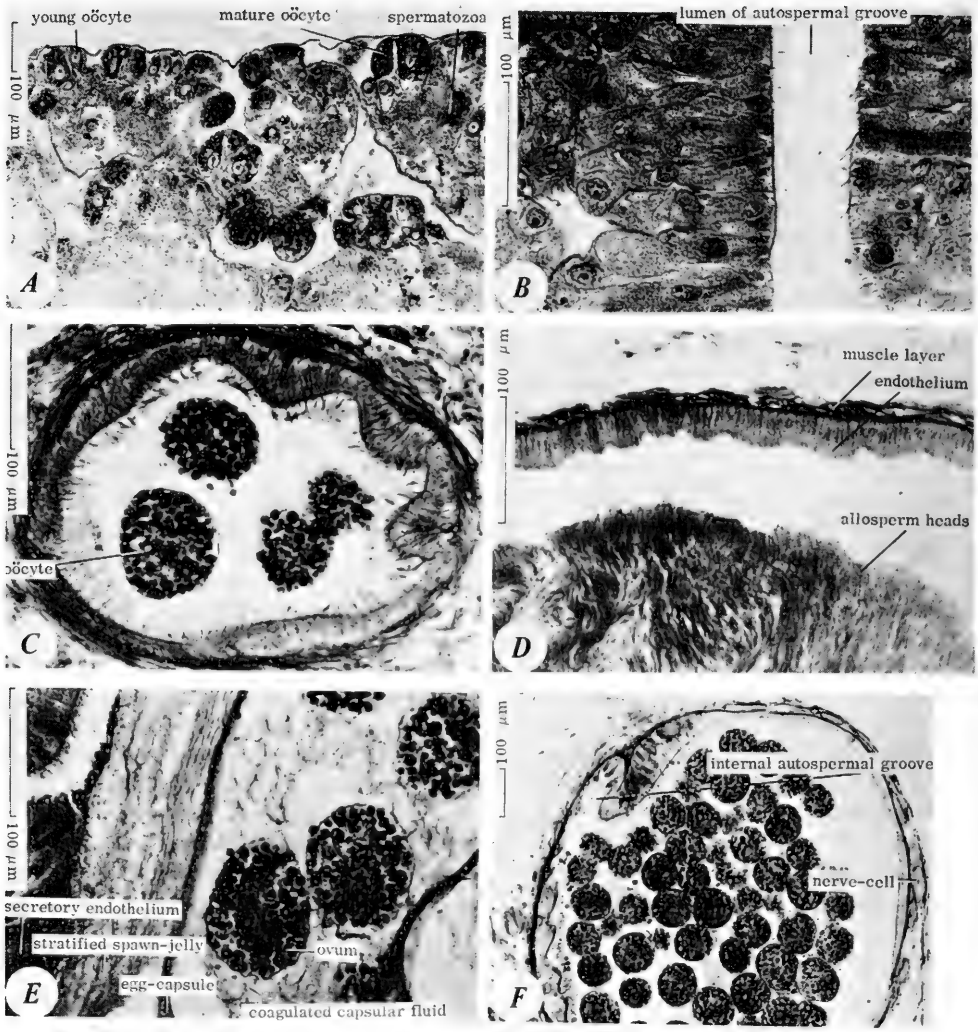


PLATE 1. Photomicrographs of sections through the reproductive system of *Aplysia depilans*.

A, Ovotestis; Lewitsky-saline and azan.

B, Internal autospermal groove in the wide distal hermaphrodite duct, showing the glandular prostatic endothelial cells; Bouin and azan.

C, First narrow region of the hermaphrodite duct of an ovipositing specimen; Bouin and azan.

D, Receptaculum seminis, showing allosperms orientated radially; the gap between the endothelial cells and the allosperm heads is a fixation-artifact; Bouin and azan.

E, Egg string in the female channel of the wide distal hermaphrodite duct of an ovipositing specimen, with secretions (probably adhesive) being added peripherally by the secretory endothelium; Bouin and azan.

F, Second narrow region of the hermaphrodite duct of an ovipositing specimen, showing the stream of oöcytes and the internal autospermal groove; Bouin and azan.

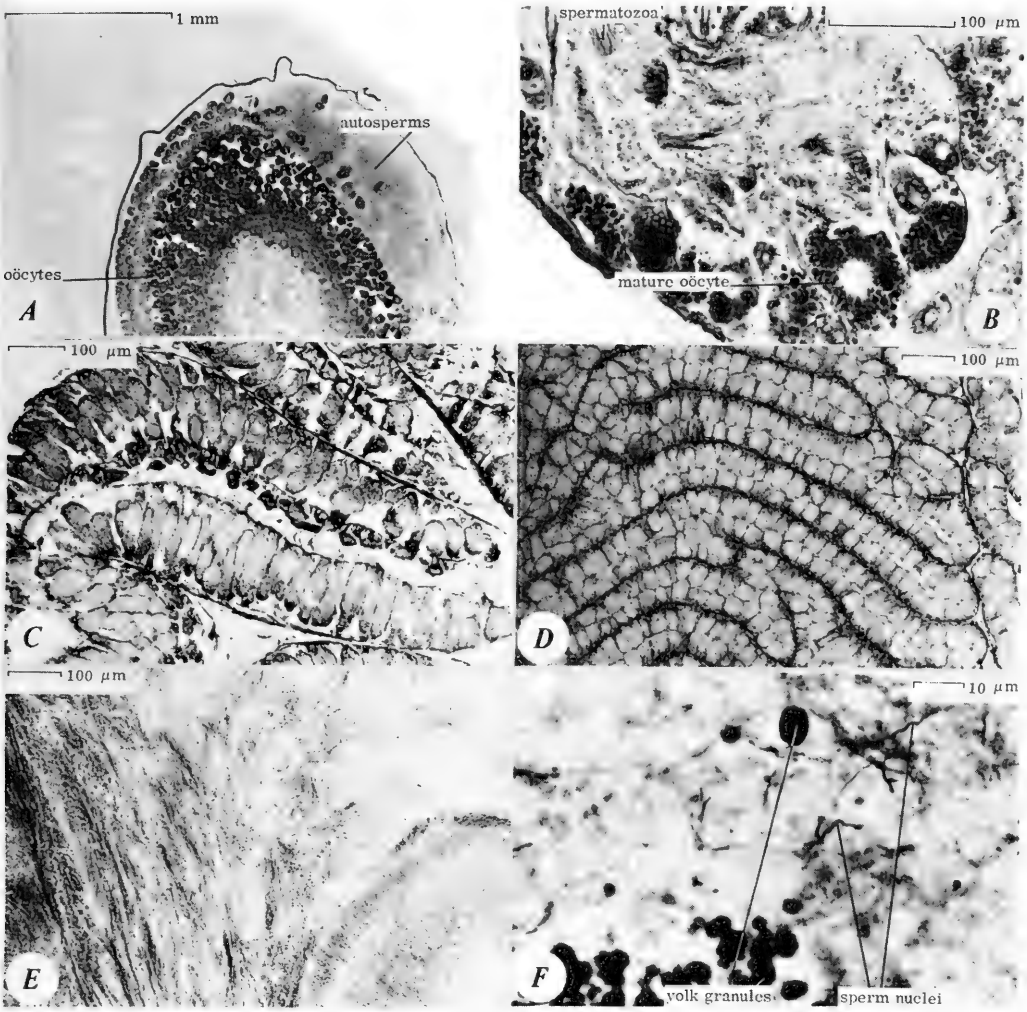


PLATE 2. Photomicrographs of sections through regions of the reproductive system of *Aplysia fasciata*.

A, Vesicula seminalis of an ovipositing specimen, showing masses of oöcytes passing through the duct, in contact with autosperms; Lewitsky-saline and azan. (The different staining reactions of oöcytes at different depths within the duct are due to the slow penetrating characteristics of the fixative and are without functional significance).

B, Ovotestis; Lewitsky-saline and azan.

C, Winding gland, showing endothelial cells in a phase of active secretion; Bouin, haemalum, and alcian blue.

D, Mucous gland; Bouin and azan.

E, Receptaculum seminis, showing swirling areas of allosperms in groups with common orientation; Bouin and azan.

F, Gametolytic gland, showing male and female gametes in various stages of digestive breakdown; Zenker-without-acetic and iron haematoxylin.

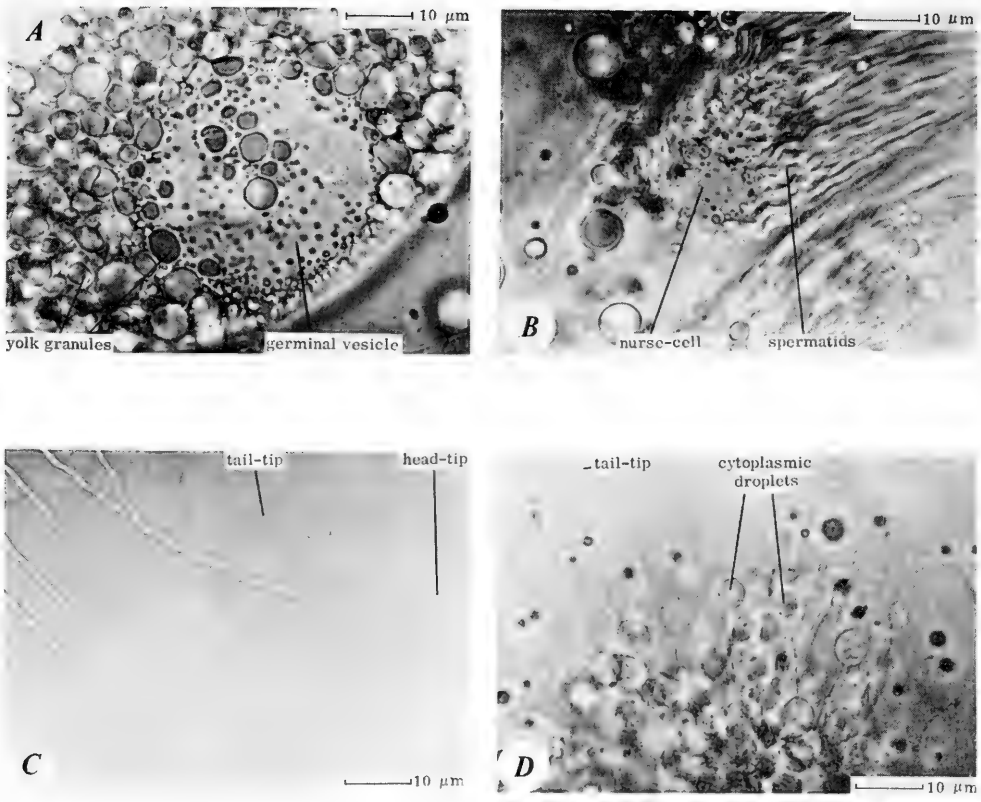


PLATE 3. Stages in oogenesis and spermiogenesis of *Aplysia punctata*; photomicrographs of live material from the ovotestis and from the vesicula seminalis.

A, Late oöcyte in the ovotestis, showing yolk granules and the germinal vesicle.

B, Late spermatids grouped around a nurse cell in the ovotestis.

C, Mature autosperms from the vesicula seminalis.

D, Late spermatids in the ovotestis, showing cytoplasmic droplets near the tail-tips.

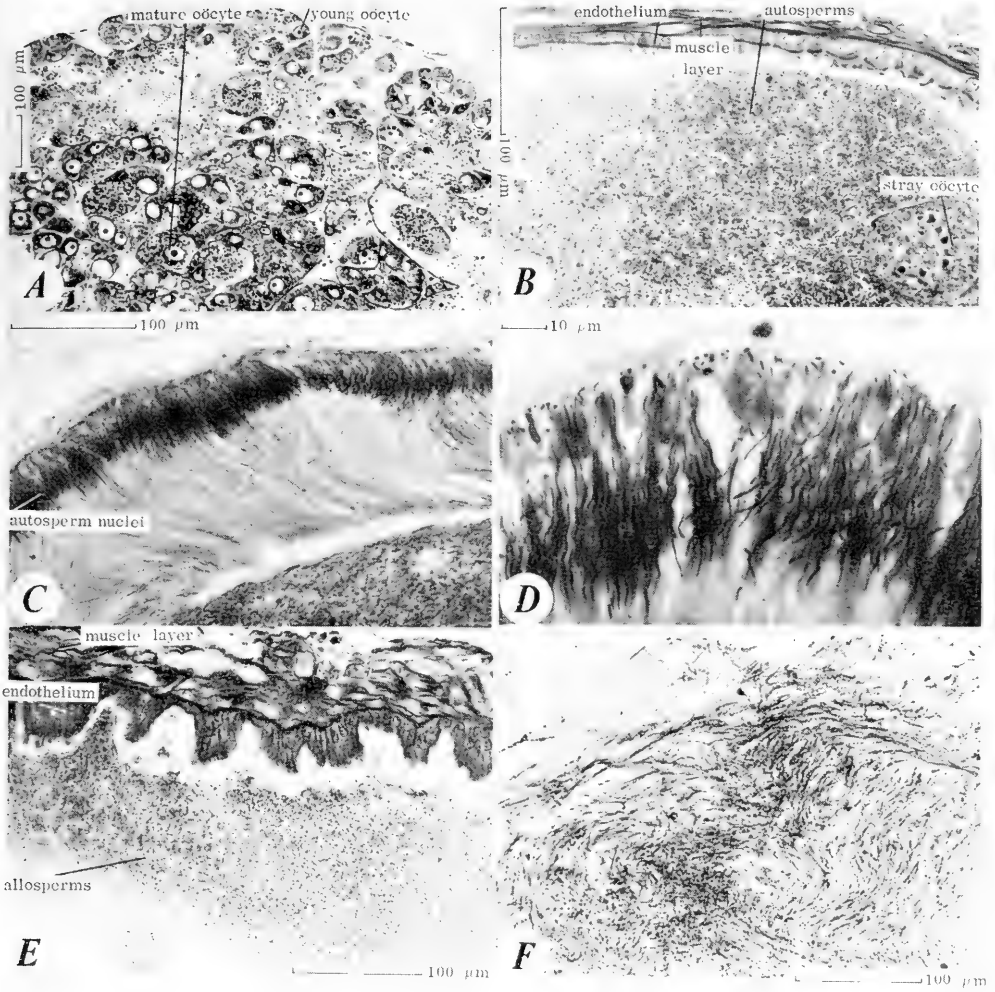


PLATE 4. Photomicrographs of sections through regions of the reproductive system of *Aplysia punctata*.

A, Ovotestis of an adult, body-length 15 cm; Lewitsky-saline and azan.

B. Vesicula seminalis of the same specimen; Lewitsky-saline and azan. In this section an oöcyte can be seen among the autosperms, but this is a rare event in a non-ovipositing individual.

C, Vesicula seminalis of another adult, showing the radial orientation of many of the autosperms; formalin and haemalum.

D, Portion of the same, at a greater magnification, showing the intimate association between the autosperm nuclei and the endothelium of the duct.

E, Receptaculum seminis of an adult, showing many allosperm heads; Lewitsky-saline and iron haematoxylin.

F, Portion of the same, at a greater magnification; the helically coiled allosperm nuclei are visible, orientated more or less at random in this region.

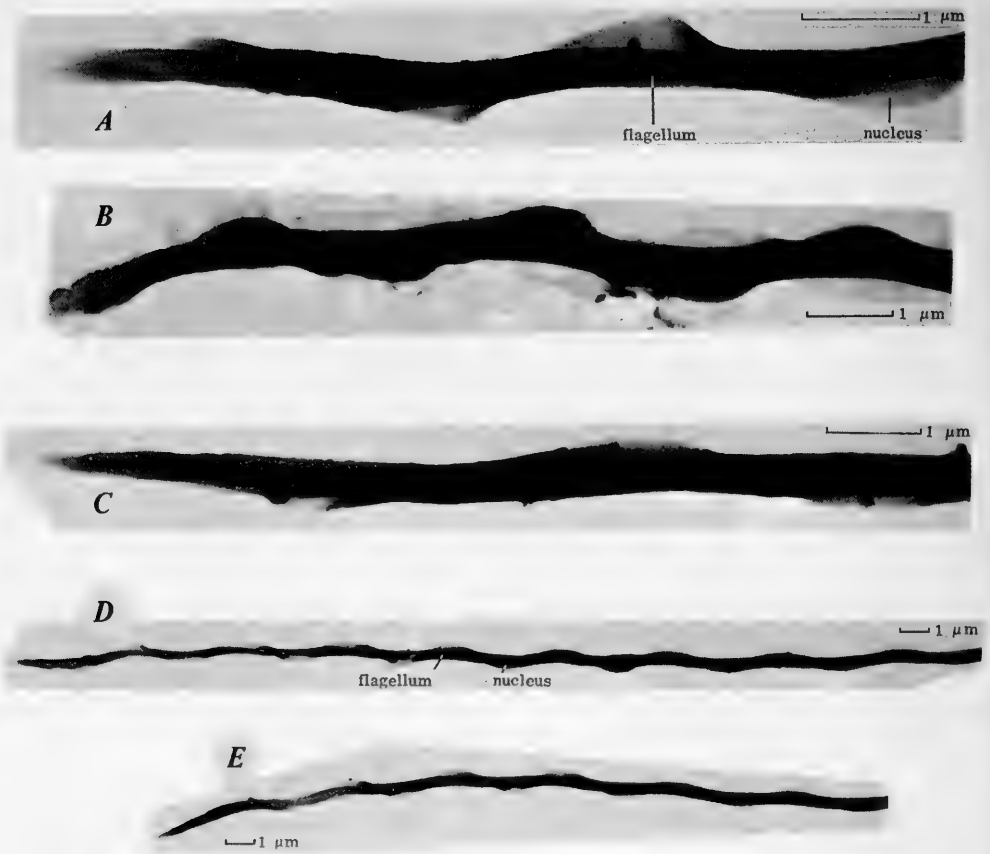


PLATE 5. Electron micrographs of autosperms of *Aplysia*, dried in osmic vapour, on collodion-coated copper grids.

A, *Aplysia fasciata*

B, *A. depilans*

C, *A. punctata*

D, E, *A. fasciata*

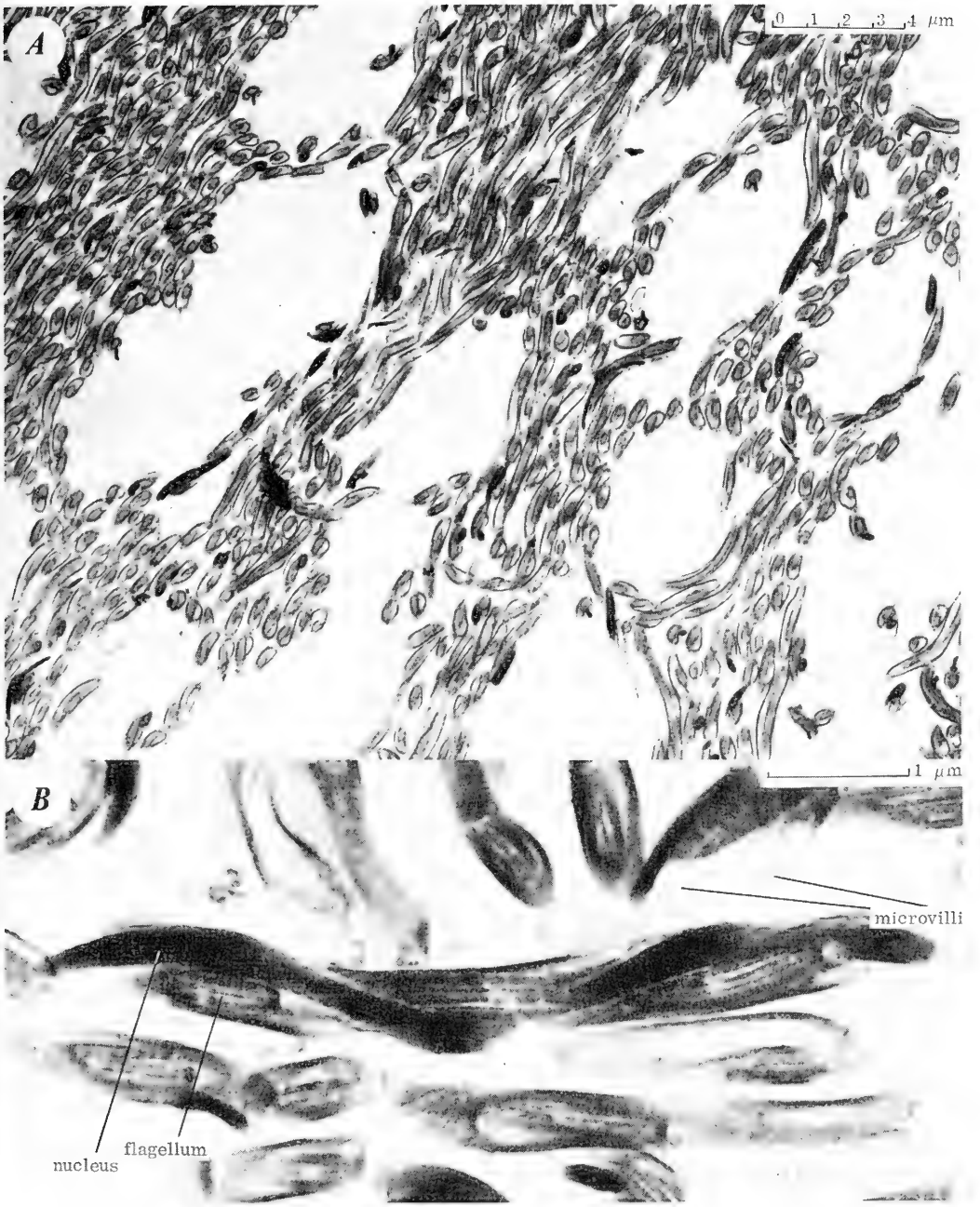


PLATE 6. Electron micrographs of sections through the receptaculum seminis of *Aplysia fasciata*.

A, Allosperms grouped around vesicles believed to anastomose, connect with the microvilli of the endothelium of the receptaculum, and serve a nutritive function.

B, Section passing through allosperms, displaying a head cut longitudinally, with 2 turns of the nuclear helix, around the axial flagellum.

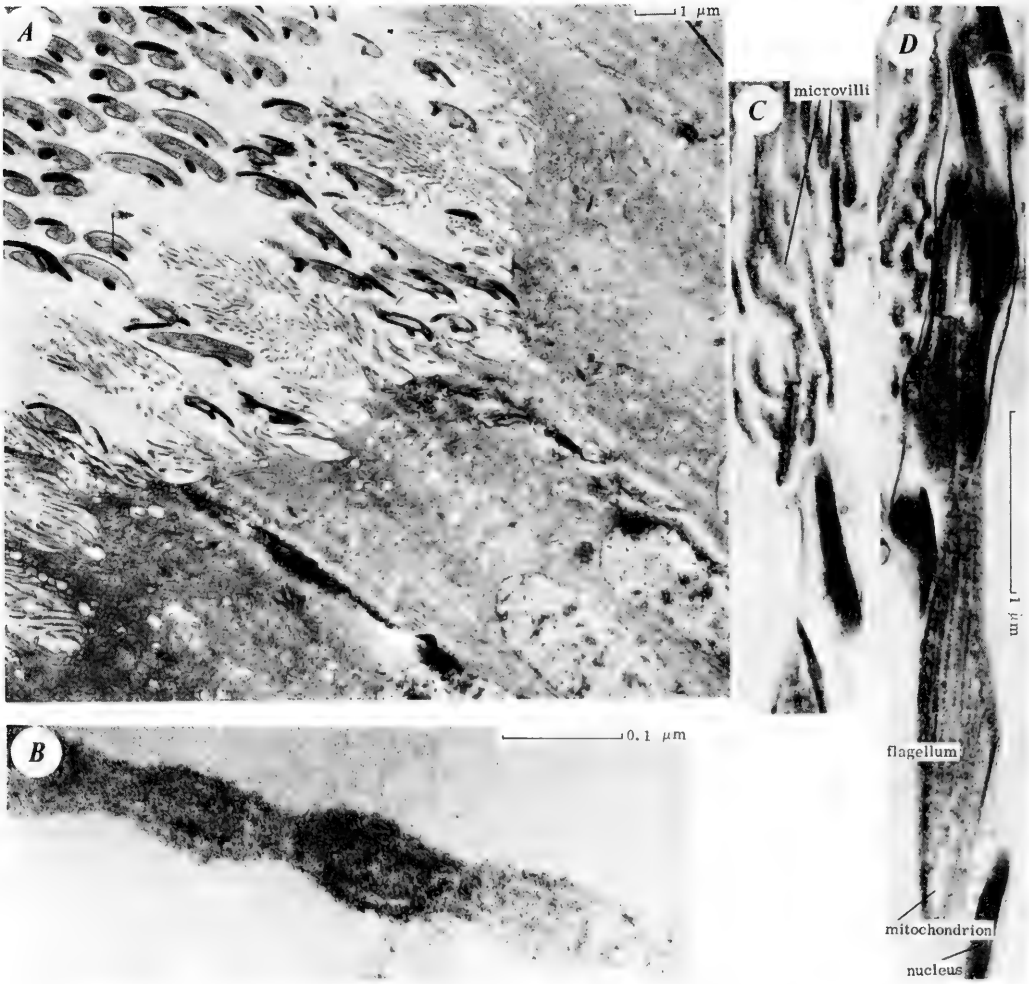


PLATE 7. Electron micrographs of sections through the receptaculum seminis of *Aplysia depilans*.

A, Orientated allosperms, facing the microvillous endothelium of the receptaculum seminis.

B, A single microvillus.

C,D, Enlargements of parts of A, showing the intimate association between the allosperm head and the endothelial microvilli.

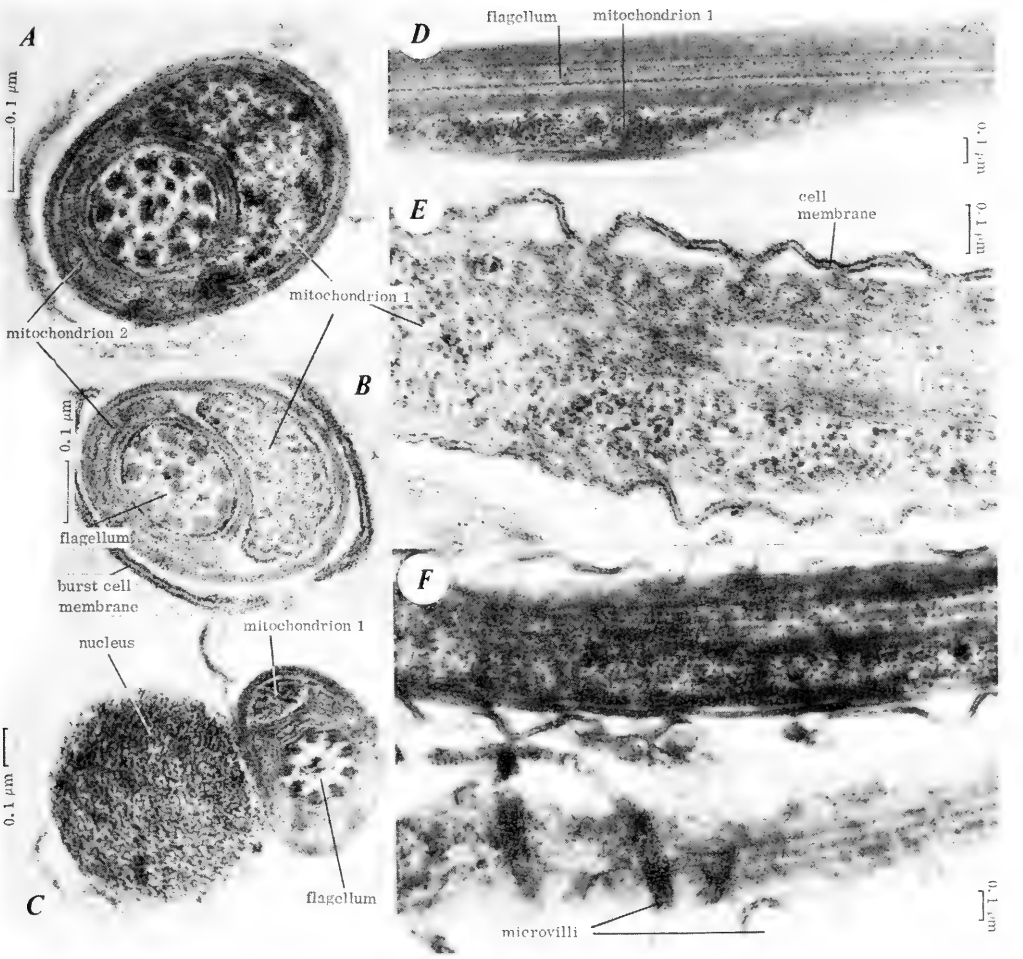


PLATE 8. Electron micrographs of sections through allosperms in the receptaculum seminis of *Aplysia punctata*.

A,B, Transverse sections through the principal-piece.

C, Transverse section through the head, showing the relative proportions of nucleus, flagellum, and mitochondria at this level.

D,E, Longitudinal section through the principal-piece.

F, Longitudinal section showing the investment of microvilli from the receptaculum seminis endothelium, believed to serve a nutritive function.

was not realized, but a number of interesting facts emerged.

First, it was established by examination that autosperms from the external autospermal groove were non-motile during copulation. Autosperms taken from the penial groove of a vivisected, copulating individual were similarly inactive. It was then clear that activation is not immediately induced solely by secretions of the male tract of the copulation donor.

Sea-water extracts of various female organs were homogenized, centrifuged, and the supernatant fluid tested with samples of non-motile autosperms from the vesicula seminalis of the same or of another individual. Tests were performed using extracts of the albumen gland, the wide distal hermaphrodite duct, gametolytic gland and virgin receptaculum. The extracts were tested in some experiments singly, in others in synchronous combination and in various sequential combinations, but in no case was genuine activation achieved. The problem remains unsolved. It seems probable that extracts prepared for eliciting autosperm activation will be effective only if the original tissue was taken from an adult which was in precisely the correct physiological state. Many more experiments will be needed to test this hypothesis.

TRANSLOCATION OF SPERMATOZOA

During copulation, which in the laboratory occurs chiefly during the day, autosperms are discharged from the vesicula seminalis (Fig. 1) by means of peristaltic muscular contractions of the wall of the duct. They are then transported by ciliary action along the internal autospermal groove (Figs. 4,F; 9,A) and the external autospermal groove (Fig. 5,H) at approximately 0.3 mm/sec. Muscular action plays no part in their translocation along the groove. The external groove is continued (Fig. 5,G) to the tip of the penis (Fig. 10), which during copulation is extended and erected by haemolymph engorgement.

Groups of individuals copulate in chains, as Eales (1921) and others have said. Each animal acts as a male (allosperm donor) to that in front, and as a female (allosperm recipient) for the individual behind in the chain. The penis enters the hermaphrodite opening of the recipient and, guided by the typhlosole of the wide distal hermaphrodite duct, reaches up to the level of the stalk of the receptaculum seminis (Fig. 10). As Eales (1921) pointed out for *Aplysia punctata*, earlier authors who believed that the penis entered the aplysiid gametolytic gland (called by various names in the older literature), are undoubtedly mistaken. The details were established for *A. depilans* by dissection and sectioning after plunging copulating specimens into boiling Bouin's fluid. This technique was not so successful using the other 2 species, in which the penis was usually withdrawn on death. *A. depilans* is the only species of those dealt with which bears spiny warts on the basal region of the penis (Fig. 10), and these grip the inside of the wide distal hermaphrodite duct of the recipient and make withdrawal difficult. Indeed, so strongly do the spines grip the duct that tears often result, and small streams of allosperms may be seen leaking into the haemocoel (Fig. 10,A).

The spermatozoa of the copulation donor leave the tip of the penis and enter the stalk of the receptaculum seminis. They are stored in this organ in the active state until they are expelled during oviposition by means of muscular contractions of the wall of the receptaculum. Their short journey to meet the stream of outgoing oöcytes is probably accomplished at that time principally by their own efforts.

OVIPOSITION

The physiological mechanism which controls the initiation of oviposition is unknown. Vicente (1966) has claimed

that oviposition and copulation can be induced by the removal of an inhibitory centre in or near the rhinophoral tentacles. We have attempted to repeat this experiment, using *Aplysia depilans* (Vicente worked with *A. rosea*, probably a variety of *A. punctata*), but without success. In our experiments, removal of the rhinophoral tentacles never led to copulatory or ovipository behaviour, but rather to the adoption of a wounded posture with the head contracted. Usually, the operated individual died within a few days, often with the penis extended, as is usual with mature moribund opisthobranchs.

At the onset of oviposition in normal individuals, multitudes of ripe oöcytes leave the ovotestis lobules (Fig. 6), generally during the hours of darkness, probably as the result of co-ordinated contractions of the delicate extrinsic musculature of the gonad. The oöcyte stream is transported by ciliary action through the hermaphrodite ductules, at a speed of 0.1 mm/sec., to the initial narrow region of the hermaphrodite duct (Pl. 1,C). They then enter the vesicula seminalis (Pl. 2,A; Figs. 4,D; 7,A and B); as they do so, they commence the first meiotic maturation division, but this is arrested after the breakdown of the germinal vesicle membrane and the assembly of the chromosomes upon the spindle equator (Figs. 4,D; 7,C). The female gametes at no stage possess the power of independent movement. The oöcyte stream is conducted through dense masses of autosperms along the tract of ciliated endothelial cells (Fig. 7,A) described earlier, travelling at a speed of 0.2 mm/sec. In vivisections of ovipositing specimens, the violet-cream oöcytes were seen clearly through the walls of the various ducts (Fig. 6). After histological fixation, the oöcytes fall away from the conducting tract of the vesicula seminalis and sections often show them lying at random in the duct (Fig. 7,A and B); this is an artifact.

The oöcyte stream travels through the 2nd narrow region of the hermaphrodite duct (Figs. 1; 7,C; Pl. 1,F) at approximately 1 mm/sec. and then passes the opening of the albumen gland, whose secretion, consisting of a viscous fluid loaded with amber secretory spherules, is poured upon them. Sections through the albumen gland of an ovipositing specimen show the secretory cells to be in the process of active secretion into the lumen of the gland (Figs. 5,E; 7,E). As this secretion is mixed with the oöcytes (Fig. 8A), they meet quantities of active physiologically mature allosperms, which have been expelled from the nearby receptaculum seminis (Fig. 1). Peristaltic contractions of this organ may be readily observed in vivisected specimens. The mixture of allosperms and ripe oöcytes is carried past the sphincter muscle into the fertilization chamber (Fig. 1); the sphincter presumably acts as a valve permitting the oöcytes to enter only singly or in small groups. From the fertilization chamber the mixture of gametes enters the convoluted winding gland through which the oöcytes travel at 2 mm/sec. (Pl. 5,C; Fig. 7,D). It is in the fertilization chamber and the winding gland that sperm-penetration commences, but the complex events of amphimixis occupy several hours, of course, and are still incomplete (Fig. 9, C) when the spawn band is expelled from the external hermaphrodite opening. The first mucous secretions are added to the gamete stream as it passes through the winding gland (Fig. 7,D).

The duct then widens to form the mucous gland section of the efferent female passage (Figs. 1; 6). Jelly is copiously added, forming at first loose mucous globules (Fig. 8,A), but sections passing through more distal parts of the mucous gland (Fig. 8,B) show that the egg-capsules have been formed, and the mucus forms a stratified coat around the egg stream.

The egg-capsules (Fig. 8,B) each enclose up to 50-60 ova, depending on the species, and in sections are seen to be approximately 4μm thick. The mecha-

nism of their formation is not known, but it is certain that they are not formed by the ova themselves, nor, as was at one time thought, solely by the secretions of the albumen gland. The egg-stream proceeds through the coils of the mucous gland at a speed of approximately 0.5 mm/sec. and is virtually complete as it leaves the mucous gland (Fig. 8,B) and enters the final region of the efferent tract, the wide distal hermaphrodite duct (Figs. 2,A; 6).

As the spawn passes through the female channel of the wide distal hermaphrodite duct, guided by the great typhlosole, it receives the final copious secretion of the glandular endothelial cells (Pl. 1,E; Fig. 9,A). It is probable that this final secretion is an adhesive one, facilitating the attachment of the spawn-ribbon to the substratum. Certainly, the spawn is very tacky as it emerges from the external hermaphrodite opening and passes down the external autospermal groove to the ground. The parent does not seem to exert any pressure upon the spawn as it reaches the substratum. Spawn emerges at approximately 30 cm/hr., and forms a tangled mass, as the parent moves slightly from time to time.

Moving allosperms may be detected in the spawn jelly or in the capsular fluid in spawn removed from the wide hermaphrodite duct, and for some hours after oviposition. The gradual engulfment of the sperm by the ovum was observed in live material. The final stages of egg maturation, with the production of polar bodies, commence within a few hours of oviposition.

The number of eggs per mass and some other details vary between the species, as shown in Table 2. Illustrations of the spawn of the 3 species are given by Bebbington & Thompson (1968).

ARTIFICIAL FERTILIZATIONS

Naked eggs taken from the efferent stream within the vesicula seminalis of a vivisected spawning specimen were mixed with allosperms from the same or from a different individual. A high proportion of successful fertilizations in *Aplysia fasciata* and *A. depilans* were obtained and the embryos reared through the early cleavages. Although all died before gastrulation, the technique is clearly of potential utility in studies of the functional morphology of the opisthosbranch sperm. This is the first time successful artificial fertilizations have been reported for an internally fertilizing gastropod.

CONCLUSIONS

Many features of the aplysiid reproductive system remain obscure:

1. The mechanism of allosperm activation following copulation is still uncertain, although it has been shown in the present paper that the prostatic secretion alone is insufficient. Aqueous extracts of various parts of the female tract have also proved ineffective, and it is clear that these organs must be treated in precisely the phase during which they are normally active.

2. The endocrine control of oviposition and copulation is still far from clear. Vicente's (1966) claim that these behavioural phases are controlled from an inhibitory centre situated in the rhinophoral areas is inadequate to explain, for instance, the co-ordinated expulsion of oöcytes from the ovotestis which initiates oviposition.

3. The means by which allosperms from the receptaculum seminis are guided to the oöcyte-stream during oviposition are not understood. While the suggestion is made in the present paper that this last short journey is accomplished by the efforts of the motile gametes themselves, this is by no means conclusively established.

4. The mechanism of the build-up of the egg-capsules is not clear. Ghiselin (1965) is incorrect in claiming that the capsules are formed in the winding gland; the capsules make their appearance while the oöcytes travel

through the most distal regions of the the mucous gland. The parts played in capsule formation by the secretions of the albumen gland, winding gland and mucous gland are not precisely understood.

5. The means by which stray gametes are conducted to the gametolytic gland are unknown. Furthermore, it is not known whether the male gametes found in the gametolytic gland are autosperms, or allosperms, or both.

6. The functional morphology of the aplysiid spermatozoon during penetration of the oöcyte presents a number of incompletely understood features. While the suggestion is made in this paper that the helical form of the aplysiid male gamete functions to bring about a helical path of progression (as in dorid nudibranchs, Thompson, 1966), the adaptive significance of this mode of progression is not known. Engulfment of the spermatozoon by the oöcyte does not result from the flagellation of the male gamete, and the evidence suggests that sperm-motility is of use during the stage immediately before gamete-contact is established.

ACKNOWLEDGEMENTS

It is a pleasure to record our indebtedness to Professors Robert Weill and G. M. Hughes, for the provision of laboratory facilities, and to Drs. M. A. Sleigh and A. E. Dorey, for helpful advice in techniques of electron microscopy. We are grateful for the help we received from the Directors and research staff during working visits to the marine laboratories at Plymouth, Port Erin and Menai Bridge.

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RÉSUMÉ

STRUCTURE ET FONCTION DES ORGANES REPRODUCTEURS DE 3 ESPÈCES D'*APLYSIA* (GASTROPODA - OPISTHOBRANCHIA)

T. E. Thompson et A. Bebbington

Les appareils reproducteurs de trois espèces d'aplysies montrent une séparation incomplète des canaux efférents pour les gamètes mâles et femelles. Le système fonctionne de façon à déplacer les ovocytes (par action ciliaire) pendant la ponte, à expulser l'autosperme (par action ciliaire et musculaire), et à recevoir l'allosperme transmis pendant la copulation. Des typhosoles et des appareils ciliaires complexes servent à séparer ces différentes activités; les fonctions des septa et des canaux efférents du conduit hermaphrodite commun sont décrits. La progression graduelle des émissions gamétiques a été suivie sur des sections sériées à travers l'ensemble du complexe de la glande nidamentaire, sur des spécimens tués lors des différentes phases d'activité. Les voies efférentes des gamètes femelles pendant la ponte et la position du pénis pendant la copulation, ont été élucidés. L'autofécondation est évitée par la rétention de l'autosperme en état d'immaturité physiologique, à un stade non mobile, de telle sorte que la chaine efférente d'ovocytes lors de la ponte, rencontre de l'autosperme immature, et que la fécondation a lieu par l'allosperme actif du réceptacle séminal. L'autofécondation artificielle peut être réalisée si un échange séminal est possible avant que les gamètes ne soient mélangés. L'activation des spermatozoïdes advient seulement après leur transfert lors de la copulation; cette activation est réalisée pour les sécrétions du tractus femelle et de la poche copulatrice, mais des tentatives pour isoler l'agent de l'activation ont été vaines.

Les précédents comptes-rendus sur la spermatogénèse et la structure des spermatozoïdes mûrs sont incorrects à bien des égards. Le noyau des spermatozoïdes des aplysies a une structure hélicoïdale et l'hélice mitochondriale traverse de bout en bout la tête avec le flagelle qui prend naissance tout près de l'extrêmité antérieure du gamète. La structure du spermatozoïde et du réceptacle séminal a été examinée au microscope électronique. On démontre que la spermathèque fonctionne uniquement pour digérer les gamètes perdus et l'on propose de l'appeler la glande gamétolytique.

RESUMEN

ESTRUCTURA Y FUNCION DE LOS ORGANOS REPRODUCTORES DE TRES ESPECIES DE *APLYSIA* (GASTROPODA: OPISTOBRANCHIA)

Thompson y Bebbington

El tracto reproductor de tres especies de gastrópodos aplysidos muestra separación incompleta de los canales eferentes para las gametas masculina y femenina. El sistema funciona de tal manera que desplaza oocitos (por acción ciliar) durante la puesta, expulsa autoesperma (por acción ciliar y muscular) y recibe aloesperma transferido durante la fecundación en cadena. Tractos ciliares complejos y typhlosoles sirven para separar varias actividades; se describen las funciones de los septa y los canales eferentes del ducto hermafrodita común. La formación gradual de la masa ovigera fué observada en una serie de cortes de todo el complejo nidal, en ejemplares muertos en varias fases de esa actividad. El pasaje eferente de gametas femeninas durante la puesta, y la colocación del pene durante la cópula, fueron elucidadas. Autofertilización está prevenida por la retención de autoesperma en un estado inmóvil fisiologicamente inmaduro, de modo que la corriente eferente de oocitos durante la ovoposición encuentra autoespermas no maduros, y la fertilización se produce por aloespermas activos del receptáculo seminal. Autofertilización artificial puede ser inducida si se permite intercambio seminal antes de que las gametas se mezclen. Activación de los espermatozoos ocurre solamente antes de su intercambio durante la cópula; esta acción es llevada a cabo por la secreción del tracto femenino del copulador recipiente, pero no se logró aislar al agente activante.

La información que se tenía sobre espermogenesis y sobre la estructura del espermatozoo maduro, eran incorrectas en muchos aspectos. El núcleo del espermatozoo aplysido, tiene estructura helicoidal, y el par de mitocondrias dispuesto helicoidalmente circula en la cabeza con el flagelo, que se origina cerca de la punta anterior de la gameta. La estructura del espermatozoo y de los órganos acumuladores de esperma se investigó por medio del microscopio electrónico. La espermateca funciona solamente para ingerir las gametas sueltas y perdidas, y se propone sea llamada "glándula gametolítica".

АБСТРАКТ

СТРУКТУРА И ФУНКЦИЯ ОРАГАНОВ РАЗМНОЖЕНИЯ ТРЁХ ВИДОВ *APLYSIA* (GASTROPODA: OPISTHOBRANCHIA)

Т.Е.ТОМПСОН И А.БЕББИНГТОН

Половые пути трёх видов аплизий имеют неполное разделение выводных протоков для мужских и женских гамет. Система функционирует так, чтобы перемещать ооциты (с помощью действия ресничек) во время откладки яиц, выносить собственную сперму (действием ресничек и мускулатуры) и принимать чужие сперматозоиды, полученные при перекрестной копуляции. Сложные ресничные тракты и тифлозоли служат для разобщения этих различных действий, описаны функции септы и выносящих путей общего гермафродитного протока. Постепенное формирование кладки просматривалось на сериальных срезах на протяжении всего комплекса нидаментальной железы у особей, убитых на различных стадиях активности. Были выяснены процесс выноса женских гамет во время откладки яиц и положение пениса во время копуляции. Самооплодотворение предупреждается задержкой собственных сперматозоидов в физиологически-незрелом, неподвижном состоянии, так, что с потоком выносимых ооцитов при откладке яиц встречаются незрелые спермии, и оплотворение

осуществляется активными чужими спермиями из семеприемника. Искусственное самооплодотворение может быть вызвано, если сделать возможным обмен спермой до смешения гамет. Активация сперматозоидов происходит только после их обмена во время копуляции; эта активация осуществляется с помощью секреции женского тракта партнера-реципиента, но попытки изолировать активирующий агент были безуспешны. Существовавшее прежде мнение о спермиогенезе и о структуре зрелых спермиев во многих отношениях неправильны. Ядро спермия аплизиид имеет спиральную структуру, и спирально-расположенная пара митохондриальных тяжей проходит в головку вместе со жгутиком, который возникает вблизи переднего конца гаметы. Структура спермия и хранящих сперму органов изучались с помощью электронного микроскопа. Показано, что сперматека функционирует только для переваривания блуждающих гамет и было предложено называть её гаметолитической железой.

MALACOLOGIA, 1969, 7(2-3): 381-396

NUCINELLA SERREI LAMY (BIVALVIA: PROTOBRANCHIA), A MONOMYARIAN SOLEMYID AND POSSIBLE LIVING ACTINODONT[1]

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ABSTRACT

Specimens of *Nucinella serrei* Lamy obtained off North Carolina, U.S.A. (Lat. 34° 16. 6'N: Long. 75° 68.6'W) at a depth of 450 metres have allowed the first morphological description of the soft parts of a member of the family Nucinellidae.

Nucinella serrei is unique in that it is the first monomyarian bivalve to be described in which the posterior adductor muscle, and not the anterior, is absent. Furthermore, the enlarged single anterior adductor muscle remains anterior in position. The lack of the posterior adductor muscle is compensated for by the extreme posterior position of the opisthodetic ligament, the well developed posterior pedal retractor muscles and the long anterior lateral tooth. The latter prevents the sagittal sheering of the 2 valves. The ligament and pedal retractor provide the posterior attachment of the 2 valves.

Analysis of its morphology shows that *Nucinella serrei* is a protobranch bivalve and that it is closely related to the Solemyidae. The gills are very similar to those of *Solemya* and very small non-ridged palps are present. The large foot has a longitudinally divided sole which is fringed with large papillae. It is not directed forwards as it is in *Solemya* but is held in the same position and attitude as the foot of *Nucula*. There is a pair of well developed statocysts dorsal and lateral to the pedal ganglia.

The gut of *Nucinella serrei* is simple and resembles that of *Solemya*. The stomach is chitin-lined except for a narrow ventral groove that leads by way of a single aperture to the digestive diverticula. The groove continues posteriorly to the aperture as the only rejectory tract leading to the mid-gut. The short style sac is combined with the mid-gut. The digestive diverticula open to the right and left from a single "vestibule" that is positioned anteroventrally to the aperture from the stomach. On each side the diverticula are divided anteriorly into a sac-like part and posteriorly into 4 finger-like tubules each with a very narrow lumen.

The heart of *Nucinella serrei* is large and the blood system very extensive. A complex of blood sinuses occupies much of the body cavity.

It is suggested that there is the possibility that *Nucinella serrei* may be derived from the actinodont bivalves of the Lower Ordovician and that the simple gut, small palps and large gills represent the primitive condition in the (protobranch) bivalve rather than the complex gut and palps of recent nuculids.

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Since the original description of the Eocene fossil *Nucinella miliaris* by Deshayes (1829) it has been realized that this is a particularly difficult genus to place within the classification of the Bivalvia. Volks (1956), who gives a comprehensive account of the family Nucinellidae and lists all the known literature on the group, offers no comment as to the position of this family within the Bivalvia. Theile (1935), from hinge characters, included the genus *Pleurodon* (*Nucinella*) in the taxodont superfamily Arcacea. Other authors, e.g., Smith (1885), have linked the genus with the protobranchs.

Some 21 fossil species are recognized (Volks, 1956). These belong to the genera *Manzanella* and *Nucinella*, the latter including the sub-genus *Huxleyia*. The earliest known fossil is *Manzanella elliptica* Girty from the Permian of New Mexico. Twelve recent species of the genus *Nucinella* have been recorded from widely separated localities in the Atlantic and Pacific in depths ranging from 10 to 470 metres (Volks, 1956). So far, only shell characters have been described.

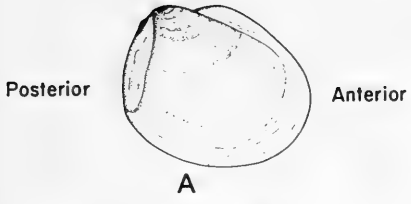
In the past the main reasons for not including the genus *Nucinella* in the Protobranchia has been its external ligament and its unusual hinge structure. The latter may bear as many as 8 transverse cardinal teeth arranged on each side of the umbo. In addition to the cardinal teeth, there is at least 1 long lateral tooth in each valve. Apart from these features, shell shape and colour closely resemble those of the genus *Nucula*.

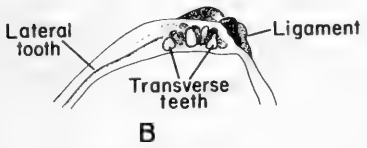
Through the kindness of Dr. F. Grassle of the Duke Marine Laboratory, North Carolina, U.S.A., we were able to examine *Nucinella serrei* Lamy. The specimens were taken from the Duke Laboratory station 349 (Lat. 34° 16.6'N, Long. 75° 48.6'W) at a depth of 450 metres, using a Van Veen grab. While the following observations form part of work in progress towards a definitive monograph on the Protobranchia, we are of the opinion that these observations are of sufficient importance to warrant a separate and early description.

THE SHELL

When we first saw in 1965 a single specimen of, to us, an unknown bivalve, our first reaction was that this was a *Nucula* with an external ligament (Fig. 1). At that time we were reluctant to do more than examine the externals of the fragile specimen that measured but 1 mm total length. However, when in 1966 Dr. Grassle was able to provide a few more specimens, the largest measuring 3 mm total length, examination of the hinge showed clearly that it was a nucinellid, and furthermore, the animal itself showed that it was very closely related to the Solemyidae. Two recent species of *Nucinella* have been recorded from the West Atlantic: *N. adamsi* (Dall) from the Straits of Florida in 375 metres (Dall, 1889, 1898), and *N. serrei* Lamy from shell sand of Bahia, Brazil (Lamy, 1912). Comparison of the North Carolina specimens with the descriptions and specimens of the West Atlantic species and other Recent species showed that they most closely resembled *N. serrei*. In the Nucinellidae the number of cardinal teeth in each valve varies from 4 to 8. In the subgenus *Nucinella* these are arranged in 2 series, one anterior and the other posterior to the umbo. In *N. serrei* and in the present specimens the division into 2 series is not distinct. In paratype specimens[2] of *N. serrei* measuring 2.5 mm total length, there are 5 teeth in each valve (Fig. 2), the most anterior tooth being very small and little more than a slight swelling on the hinge plate. In the smallest specimen from North Carolina (1.5 mm total length) there are 4 teeth in each valve and in the largest specimen (3 mm total length) there is a small anterior 5th tooth on the left valve. It must be assumed that, as in the case of *Nucula* (Allen, 1954), additional teeth are formed as the animal grows. The other difference between the present specimens and the description of *N. serrei* is that the latter spe-

[2]Museum National d'Histoire Naturelle, Paris.





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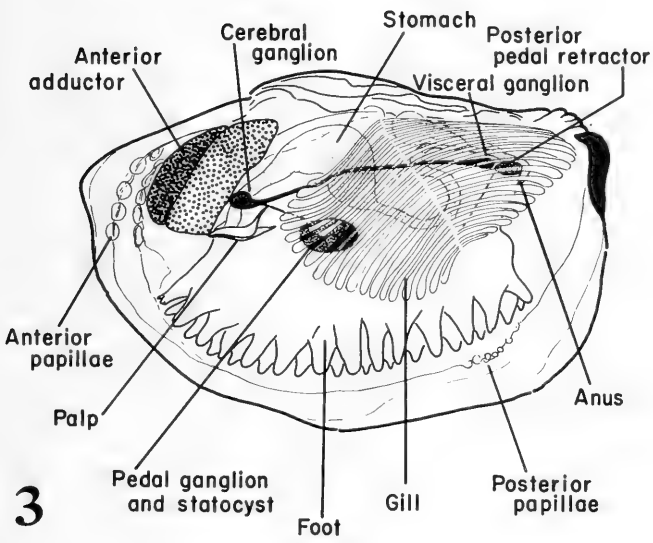

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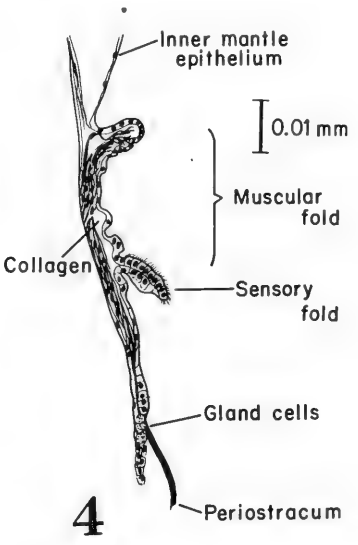

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FIG. 1. (a) External view of the right valve of a specimen of *Nucinella serrei* from 450 metres off North Carolina. Total length of specimen 1.5 mm. (b) Details of the hinge plate of the right valve showing 4 transverse teeth. (c) Details of left (lower figures) and right (upper figure) hinge plates from an angle to silhouette the various teeth.

FIG. 2. Details of the hinge region of the valves of a paratype of *Nucinella serrei*. Left valve above, right valve below. Total length of the specimen 2.5 mm.

FIG. 3. View of the left side of *Nucinella serrei* to show various details of its anatomy.

FIG. 4. Transverse section of the mantle edge of *Nucinella serrei*.

cies is shown as having a slight flattening of the postero-ventral edge of the shell (Lamy, 1912, fig. 1). However, examination of the paratypes shows that this flattening is present only in the largest specimens and is not as marked as that drawn by Lamy (1912). The smaller paratypes are similar to the specimens from North Carolina (Figs. 1 and 2).

One of the distinguishing characters of the subgenus *Huxleyia* is the possession of a single series of cardinal teeth, a 2nd is that, unlike *Nucinella*, the ligament joins the hinge plate internally. The junction of the external ligament and the shell valves in *N. serrei* is inset into the outer surface of the hinge plate (Figs. 1 and 2). It is a matter of opinion whether the characters separating the 2 subgenera are sufficiently clear-cut to make the distinction.

As mentioned above, the external appearance of the shell resembles that of *Nucula*. It is markedly inequilateral, more so than most species of *Nucula*, the part anterior to the well defined umbos being very much greater than that posterior.[3] Although the shell is thin and fragile the ventral edge is well calcified (cf. *Solemya*). The periostracum is a deep olive yellow colour typical of many protobranchs and very glossy, although it can be dark brown in preserved specimens. The antero-dorsal edge is arched. The 4 transverse cardinal teeth are of varying shapes (Fig. 1), but none are of the typical chevron shape common to most protobranchs. They lie anterior to the umbone. The hinge is further extended by an extremely long anterior lateral tooth which reaches the posterior limit of the anterior adductor muscle.

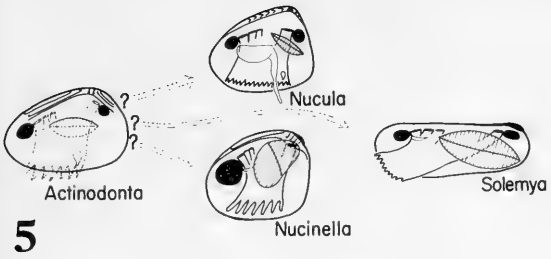
The ligament lies posterior to the umbos, that is, opisthodetic, and consists of inner and outer layers. There is no secondary extension either by fusion layer or fused periostracum. Although the ligament of *Nucinella* is basically similar to that of *Solemya* (Owen, 1959), there is no extension of the anterior layer related to the anterthe anterior layer related to the anterior elongation of the shell such as octhe elongate anterior lateral tooth of *Nucinella*, which is not present in *Solemya*, serves the same functional purpose as the anterior outer layer in *Solemya* in preventing a sheering movement at the hinge.

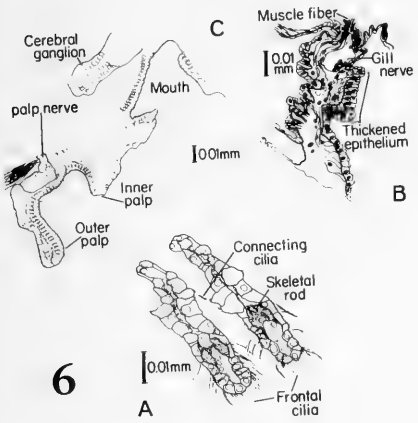
THE MANTLE AND ASSOCIATED ORGANS[4]

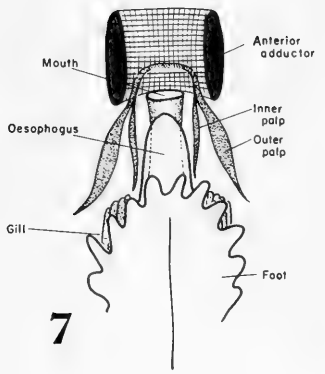
The mantle of *Nucinella serrei* is one of the least specialized of any described bivalve. There is no mantle fusion and neither a posterior inhalent nor exhalent aperture is formed (Fig. 3). This is markedly different from the species of *Solemya* in which there is extensive ventral fusion of the inner muscular folds which separates the exhalent aperture from the pedal/inhalent aperture (Orton, 1913; Yonge, 1939; Owen, 1956, 1961). Typically, there are 3 folds at the edge of the mantle of *Nucinella*. The inner, muscular fold is relatively deep and well developed - more so than in *Nucula* - and it is these muscles in *Solemya* that are particularly strongly developed and which when contracted cause the non-calcified ventral shell margins to be drawn into the mantle cavity. In addition to the muscle there is a considerable amount of collagen to the inside of the muscle fibres beneath

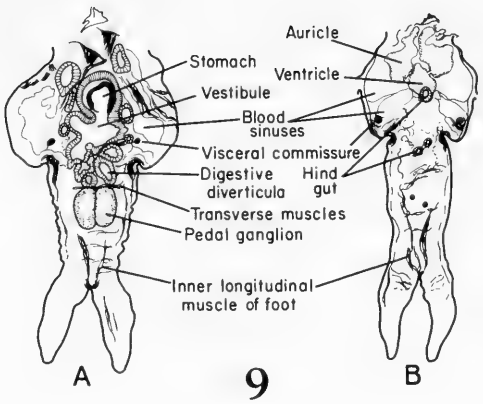
[3] In the original and subsequent descriptions of species of *Nucinella*, the shell has been wrongly orientated so that the anterior end has been thought posterior and vice versa. Volks (1956) expressed some doubt as to the orientation and this is now amply confirmed.

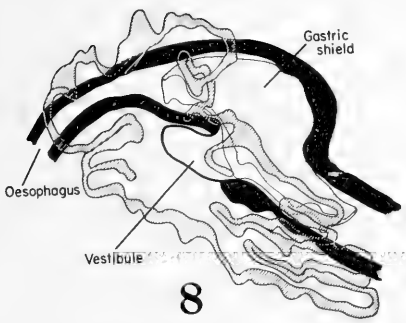
[4] The morphology of the soft parts was investigated by means of whole mounts and serial sections. The whole mounts were stained in Erhlich's haemotoxylin. Excess stain was removed by immersion in 70% acid alcohol so that on subsequent "bluing" only nuclei remained stained. This technique is effective in that it gives the impression of a 3 dimensional line and dot figure. Sections of decalcified specimens with periostracum and ligament remaining, were cut at 10u thickness and stained with Aluminium Methylene Blue, Haemotoxylin and Eosin and in Azan triple stain. Reconstructions of organs were made from detailed accurate drawings of sections, using a Wild microscope with drawing attachment.











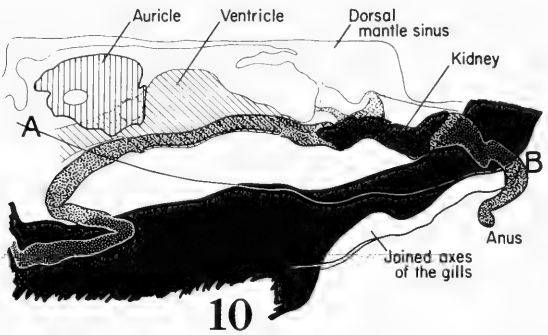


FIG. 5. Differences in the morphological features of several bivalve molluscs and possible evolutionary trends from a hypothetical actinodont.

FIG. 6. *Nucinella serrei*. (a) Transverse section of 2 adjacent gill filaments. (b) Transverse section of the left gill axis. (c) Transverse section through the mouth and left palp.

FIG. 7. Semidiagrammatic view of the region of the mouth and palps of *Nucinella serrei* as viewed from the ventral side.

FIG. 8. Lateral view of stomach and digestive glands of *Nucinella serrei* as reconstructed from serial sections. The digestive diverticula of the left side is outlined by cross hatching. The gastric shield is stippled and the gut is outlined in solid black.

FIG. 9. Semidiagrammatic drawings of transverse sections of *Nucinella serrei* (a) through the stomach and (b) through the heart. The muscles of the foot are not shown in any detail. The dorso-ventral length of the 2 sections is approximately 1 mm.

FIG. 10. Lateral view region of the heart and kidney of *Nucinella serrei* reconstructed from serial sections. The black area represents pedal musculature. The line of attachment of the gill axis (A - B) with the body is shown, as is also the course of the hind gut.

the inner epithelium (Fig. 4). The middle sensory fold is a simple flap, the upper surface of which is ciliated. Anteriorly close to the anterior adductor muscle the sensory fold is modified to form 4 or 5 simple papillae (Fig. 3). The papillae are dome shaped structures the epithelial cells of which appear to be indistinguishable from the remainder of the sensory fold. Posteroventrally at the level of the heel of the foot there is a second series of 4 to 6 papillae. These are smaller than those anterior but are of a similar simple structure. The papillae are almost certainly situated where the inhalent current enters anteriorly and the pseudofaeces, and possibly the faeces, are expelled posteriorly. The papillae are homologous to those described in various species of *Solemya* (Orton, 1913; Yonge, 1939; Owen, 1959, 1961) except that in the latter the papillae are far more numerous and much larger. The periostracal groove is not found at the junction between the sensory and outer fold but close to the edge of the outer fold. The cells secreting the outer layer of the periostracum form a band 2 or 3 cells deep, parallel to and some 5 cells in from the edge of the mantle. These gland cells lie internal to the mantle epithelium at the inner end of the periostracal groove. They have fine granular contents that stain pink in Heidenheim's Azan stain (Fig. 4). The epithelial cells of the inner surface of the outer fold below the periostracal groove lay down the inner layer(s) of the periostracum (Dunachie, 1963). There is no special development of gland cells, other than isolated mucous glands, in the mantle internal to the inner mantle fold and opposite the position of the entry of the anterior inhalent current such as has been described in the more primitive eulamellibranchs (Allen, 1968).

There is no posterior adductor muscle in *Nucinella serrei*. The anterior adductor is large; it is *not*, as is the remaining posterior adductor in other monomyarians, central in position within the mantle cavity (Yonge, 1953) (Fig. 5). It lies anterior close to the margin of the mantle. In other monomyarians the adductor is so placed that it is in direct line between hinge and the position of maximum shell gape. The mechanical disadvantage of non-alignment in the case of *Nucinella* is offset by the long hinge and by the well developed paired posterior pedal retractor muscles which are inserted on the abfrontal edge of the hinge plate of each shell valve below the umbo (Fig. 5). Immediately below the insertions the left and right posterior pedal retractor muscles are contiguous. They have the secondary function of attaching the two valves together, although it is unlikely that they play much, or any, part in closing the shell on retraction of the foot.

Although *Nucinella* is unique in that its monomyarianism involves the loss of the posterior and not the anterior adductor, it is useful to make further comparisons with other monomyarian and heteromyarian species. In the case of lamellibranch bivalves it is thought that byssal attachment of the adult always precedes reduction and final loss of the anterior adductor and that the primitive condition is probably represented by the superfamily Arcacea which is byssally attached yet dimyarian (Yonge, 1953). In this group the inhalent current enters both anterior and posterior to the byssal attachment (Atkins, 1936). In some species, e.g., *Arca*, elongation of the animal has occurred. In addition it should be noted that in lamellibranchs the gill axis runs the length of the animal from the anterior adductor to the posterior margin of the mantle. In the heteromyarian Mytilacea, it appears that byssal attachment has been followed by changes in the proportions of the body and its position in relation to the mantle shell. Thus in *Mytilus* there is elevation and enlargement of the main posterior inhalent and exhalent regions and a considerable change in the position of the main axis of the body (Fig. 5) (Yonge, 1953). There is no anterior inhalent current in *Mytilus*.

In contrast, neither *Nucinella* nor any other protobranch is byssally attached, although a so called 'byssal gland' may be present. The gill (see below) in

Nucinella is orientated so that the relatively short gill axis is placed tangentially across the posterior part of the body and does not extend to the mantle edge (Fig. 3). It can be argued that anterior elongation in *Nucinella* is advantageous in that it provides space for the extension of the gill filaments, for it is important to have a large ciliated filtering area close to the inhalent aperture (posterior in *Mytilus;* anterior in *Nucinella*). The reduced extent of the posterior region in *Nucinella* is probably a simple consequence of the lack of a posterior adductor but it may be related to the non-advantage of maintaining a large suprabranchial chamber if *all* waste can be voided in the exhalent respiratory flow. Thus in *Nucinella*, there is no barrier separating pseudofaeces from faeces and no separate exhalent aperture.

The gills of *Nucinella serrei* are similar to those of *Solemya* (Stempell, 1899; Ridewood, 1903; Orton, 1013; Morse, 1913; Atkins, 1936; and Yonge, 1939) and occupy approximately 1/3 of the lateral area of the mantle cavity (Fig. 3). As shown above, the gill axes lie across the body from the mid-dorsal region to a position close to the posterior part of the heel where the muscular part of the foot merges with the body. They do not extend beyond the body to the mantle edge, but join together immediately behind the body (see Fig. 10). The gill leaflets are narrow and very elongate, corresponding in size closely to the lamellibranch filament (Fig. 3). Between 20 and 30 leaflets extend on either side of the gill axis. The filaments posterior to the axis, i.e., the outer demibranch, extend horizontally parallel to the antero-posterior axis of the body and penetrate the relatively deep umbonal cavity which is formed below the hinge plate. Those of the inner demibranch extend antero-ventrally across the foot as far as the posterior limit of the palps. Thus, in *Nucinella* and unlike *Solemya* (Yonge, 1939), it is impossible for the leaflets of the right and left inner demibranches to be joined together posterior to the body by the interlocking of the cilia at the outer ends of the adjacent filaments. Instead, a few of the posterior leaflets of *outer* demibranchs become interlocked posterior to the body.

Except that there are no ciliated knobs on the abfrontal surfaces, the structure and ciliation of the leaflets of *Nucinella serrei* is very similar to that of *Solemya*. The leaflets are held together by small ciliary pads on the lateral surfaces of adjacent leaflets (Fig. 6a). Individual leaflets of the outer and inner demibranchs join the gill axis opposite to each other, that is, they are paired and not alternate as they are in the Malletiidae (Yonge, 1939). Few, if any, mucous cells are present, but sections do show that food is present on the frontal cilia. The arrangement of frontal, latero-frontal and lateral cilia is much the same as that in *Solemya* (Atkins, 1936), but the supporting skeletal rod is much wider and longer than other protobranchs.

Muscle fibres are present in the gill axis. The main musculature consists of a pair of muscles that extend into the dorsal part of the axis from the body wall (Fig. 6b). These are not present along the whole length but are restricted to the posterior quarter behind the point at which the branchial nerve enters the axis. The posterior part of the gill is almost certainly capable of movement, but it is a matter of conjecture whether pumping occurs in the manner of *Solemya* and other protobranchs (Yonge, 1939). There are a few transverse fibres in the axis, but no obvious longitudinal muscle strands are present similar to those recorded by Atkins (1936) for *Nuculana*.

Although the gill appears to be devoid of gland cells, for most of its length, the epithelium of the lower side of the axis is thickened and forms a ridge. The cells of this ridge are narrow and elongate and their function is obscure (Fig. 6b). They do not appear to be secretory but could possibly be chemoreceptive. No trace of a hypobranchial

gland could be found.

The palps resemble those of *Solemya* (Yonge, 1939) but, whereas in *Solemya* there is 1 flap, in *Nucinella serrei* there are a pair of small simple flap-like palps to the right and left of the mouth. The outer palp of *Nucinella* originates anterior to the mouth opposite the anterior limit of the cerebral ganglia as outgrowths of the epithelium of the anterior adductor muscle. The outer palps curve outwards and upwards and terminate where mantle and body join (Figs. 3 and 7). The inner palps originate as outgrowths of the outer palps opposite the posterior limit of the mouth approximately 120μ behind the anterior limit of the outer palp. The inner palps do not curve outwards as do the outer, but remain parallel to one another along their entire length. Thus the proximal extremities of the 2 palps are widely separate (Fig. 7).

It is possible that the palps of *Nucinella* are not entirely homologous with the palps of other bivalves for they do not appear to be extensions of the rim (lips) of the mouth. Certainly the so called inner palp is not in any way connected to the ventral side of the mouth, while the outer palps of each side join together some distance in front of the mouth. However, it may be that the inner palp is homologous with the inner ridge of the groove in *Solemya* that runs from the distal end of the single palp to the anterior side of the mouth. Yonge (1939) suggests that the ridges on either side of the groove are but reduced palp lamellae (as did Morse (1913) and Ridewood (1903) before him) and that the single palp fold is equivalent to the proboscis. There is no indication that the outer palp in *Nucinella* is a reduced palp proboscis and it may be that it too is homologous with the outer ridge of the groove in *Solemya*.

The palps of *Nucinella serrei* are not ridged on the inner face but they are very heavily ciliated and supplied with mucous glands and a large nerve (Fig. 6c). Food material is probably passed from the gill edge to the front of the mouth rather than to the side as it is in other bivalves. Well developed ventrical muscles that connect with the basement membrane of the epidermis immediately posterior to the mouth and alongside the inner palps as far as the junction of the body with the anterior part of the foot may play an important part in the manipulation of the food.

The epidermis of the anterior adductor muscle of *Nucinella serrei* is also ciliated and resembles the condition in the Lucinacea (Allen, 1958). It seems probable that, as in the case of the latter group, that incoming food in an anterior inhalent current is passed directly to the mouth by these cilia. The adductor muscle itself is unusual in that there is a well developed layer of muscle fibres beneath the epidermis that lie lengthways to the epidermis and at right angles to the main adductor strands.

GUT AND DIGESTIVE DIVERTICULA

The course taken by the gut of *Nucinella serrei* through the body is very simple (Fig. 3). A relatively long oesophagus opens into the stomach which in its turn opens to a short, combined, mid gut and style sac. The hind gut extends dorsally from the mid gut in a single loop before turning posteriorly to the anus. The hind gut passes over the pedal retractors close to their insertion on to the shell before curving down into the mantle cavity between the reflected outer demibranchs of the gill.

The arrangement of the gut of *Nucinella serrei* resembles that of *Solemya*, although the diameters of the various parts are relatively larger than those of *Solemya* (Owen, 1959, 1961; Yonge 1939). *Solemya* is remarkable for the extreme reduction in size of the gut, so much so that it seems difficult to appreciate that it alone can provide sufficient nutrient material by its digestive processes (Owen, 1961). Despite its small size, the stomach of *Solemya* is said to have

all the essential features seen in other bivalves (Owen, 1961; Yonge, 1939), including an anterior ciliated region and a dorsal hood. Its dorsal and lateral walls are covered by a cuticular or chitinous lining homologous to that described in the Nuculidae (Yonge, 1939 and Owen, 1961). The stomach of *N. serrei* is considerably more simple than this (Fig. 8). Except for a narrow ventral channel which, anteriorly, directs material from the oesophagus to the relatively enormous single aperture to the ventral floor, the stomach is completely lined by chitin. Note that in *N. serrei*, unlike *Solemya*, the chitinous lining is present anterior to the ventral aperture to the digestive diverticula. Posteriorly this channel extends from the mouth of the aperture to the hind gut. It is flanked by the typlosoles and forms the route by which rejected material passes posteriorly. From the morphology, it is clear that all material entering the mouth is passed from the aperture to the digestive diverticula. There is no dorsal hood, and there is little or no ciliated surface between the posterior limit to the oesophagus and the chitinous lining of the stomach (Fig. 8). Histologically, the chitin, and the epithelium that secretes it, appears to be identical with the gastric shield and the secreting epithelium in other bivalves.

The single aperture in the floor of the stomach of *Nucinella serrei* leads directly into the digestive diverticula. Anterior to the aperture there is a large single vestibule from which open ramifications of the diverticula to the right and left of the stomach (Fig. 8). The diverticula on each side are arranged in a similar fashion. Posterior to the vestibule there are on each side 4 finger-like tubules closely applied together with a relatively narrow lumen. These extend to the posterior limit of the style sac. Anteriorly, overlying the anterior part of the stomach and the oesophagus, the diverticula of each side are large and sac-like, and roughly divided into dorsal and ventral sections. The surface area of the walls of the anterior parts of the diverticula is extended by means of shallow pockets. This differentiation into a part with a wide lumen and a part with a narrow lumen is reminiscent of the description by Yonge (1939) of the 3 masses of the digestive diverticula of the Nuculanidae. Yonge (1939) postulates that large particles exclusively enter the first mass, the tubules of which have a lumen with a cross section twice the size of that of the 2nd and 3rd masses.

At the rim of the aperture to the digestive diverticula, the ciliated epithelium of the narrow ventral section of the stomach is replaced by a layer of brush border cells. These cells encircle the aperture to a depth of 10 or 12 cells, and face into the vestibule of the diverticula, (R.I. Fig. 9). In addition, the ridge which is formed on the floor of the vestibule at the point where the diverticula of each side come together, is also fringed with a brush border epithelium approximately 3 or 4 cells deep. This brush border epithelium strongly resembles the cells of the main ducts of other protobranchs (Owen, 1956). Thus, the vestibule perhaps should be considered as a much shortened main duct leading to the diverticula. Beyond the brush border cells, the diverticula are lined with typical tubule cells. In *Nucinella serrei* there are no cells of the type found lining the secondary ducts of other protobranch molluscs (Owen, 1956). The tubule cells of the posterior narrow finger-like tubules resemble the descriptions given by Owen (1956) for *Nucula*. The majority of the cells lining the tubules are of this same type. These cells contain numbers of granules; those granules at the distal end of the cell are large and stain bright pink in Azan preparations, while smaller granules occurring at the proximal end of the cell close to the nucleus stain a grey-blue in the same staining technique. There does not seem to be any intracellular digestion taking place in these tubules, but spherical pieces of cyto-

plasm containing large granules are present in the lumen of the tubules. These spheres appear to be cut off from the proximal ends of the cells in the manner described by Owen (1956) for *Nucula*. As in *Nucula*, there is a 2nd type of tubule cell in *N. serrei* which is much less common than that described above. This is a darkly staining flagellated cell which is triangular in shape. It usually occurs in groups of 3 or 4 which are found in various positions in the tubule. The cells that line the lumen of the anterior part of the diverticula appear to be different to those of the posterior tubules. There are few large pink staining granules, although blue staining granules are present. A great variety of cell inclusions occur, and unlike the tubule cells of the posterior region, the cells of the anterior part do seem to be used for the intracellular digestion of particles. Few cells of the 2nd type were observed in the anterior part of the digestive diverticula.

It would appear that the 2 parts of the digestive diverticula in *Nucinella serrei* have different functions. It is suggested that the anterior section with the wide lumen is concerned with intracellular digestion. The posterior section is mainly secreting, and is perhaps concerned with extracellular digestion.

The posterior tubules have well defined muscle fibres around them (Fig. 9a), and these fibres are derived from transverse muscles crossing the body at the junction of body and foot. The anterior tubules are flanked by vertical muscles which originate at the dorsal side of the body and run to the latero-ventral flanks of the body. There is some evidence of muscles actually being inserted on to the ends of the tubules, as discovered in *Solemya* (Owen, 1961), but, for the most part, the musculature runs alongside the basement membrane of the tubule cells and is inserted at the body wall. These muscles probably play an important role in the movement of material in the lumen of the diverticula.

NERVOUS SYSTEM

The nervous system of *Nucinella serrei* follows the typical protobranch bivalve plan, with 3 main pairs of ganglia; the cerebral, visceral and pedal ganglia (Fig. 3). The ganglia, as well as the commissures between them, are particularly large. The cerebral ganglia on either side of the oesophagus and above the mouth give rise to a large nerve to each palp (Fig. 7), and another nerve that supplies the anterior adductor and the mantle edge. The visceral ganglia are elongate and terminate just anterior to the dorsal insertion of the posterior pedal retracters. Long visceral ganglia, little wider than the relatively stout cerebro-visceral commissure, are commonly found in protobranch bivalves. The innervation of the gill and gill axis is derived from this commissure. The pedal ganglia are extremely large, and associated with them and to the dorsal side are a pair of statocysts similar to those found in other protobranchs (Fig. 9). The statocysts are lined with a thin pavement epithelium that is sparsely ciliated, and the lumen contains a quantity of small rod-shaped crystals of varying sizes. A pair of nerves lead posteriorly from the pedal ganglia through the centre of the foot.

FOOT

The foot of *Nucinella serrei* is similar to that of other protobranchs in that the sole is longitudinally divided and the edges are deeply papillate - more so than any other protobranch examined. Like *Solemya*, the foot of *Nucinella* is very large in relation to the remainder of the animal (Fig. 3). The outer cell walls of the epithelium of the papillae and sole are very thick, but do not appear to be ciliated. Below the epithelium there is a thin layer of vertical longitudinal muscle and, successively, there is a layer of muscles running antero-posteriorly, and a second layer of vertical longitudinal muscles before the central hydro-

coele of the foot. Transverse muscle strands cross the foot. The innermost fibres of the inner longitudinal muscles occur in regular bundles, which originate above the central groove of the foot in the mid line between opposite papillae (Fig. 7). There are relatively few mucous secreting glands in the foot, and the so called "byssal" gland of other protobranchs is not present.

Solemya is a deep burrower and an active swimmer (Drew, 1900; Owen, 1961). *S. velum* constructs a Y-shaped burrow and normally lies across the angle of the Y so that one arm becomes an inhalent channel and the other an exhalent channel (Stanley, unpublished observations). The elongate form and large anteriorly directed foot of *Solemya* is related to this habit as it is in the case of *Ensis* and *Tagelus* (Yonge, 1952). In contrast, the large foot of *Nucinella* is not turned forwards, but held more in the manner of *Nucula*, and it seems likely that *Nucinella*, although it moves actively through the bottom sediment in the same manner as *Nucula*, does not construct a burrow (Fig. 5).

HEART AND KIDNEY

The heart of *Nucinella serrei* is very large. The auricles lie dorsal and lateral to the single loop of the gut, and slightly above the level of the axis of the gill. Blood from each gill is carried in a short vessel that leads from the axis directly to the auricle (Fig. 10). The right and left auricles in turn supply the single elongate ventricle. The hind gut passes through the floor of the ventricle. Both auricles and ventricle are very thin walled. The course of the circulation from the ventricle is difficult to determine from sections, but it is clear that the blood flows through a series of thin-walled sinuses, which occupy a considerable proportion of the body space (Fig. 9). These are arranged in a similar fashion to the right and the left of the body, and are supplied from a very short anterior aorta. Posteriorly, the ventricle gives rise to a pair of short vessels on either side of the gut, and to a vertical vessel that appears to supply a large dorsal mantle sinus just below the hinge line (Fig. 10). This sinus extends forward as a small vessel to a point anterior to the auricles, where it turns ventrically and merges with the anterior sinus system of the body. This dorsal sinus is very well defined and unusual in its position; its exact function cannot be determined until living specimens are examined.

Analysis of the course of the vessels and sinuses posterior to the ventricle is complicated by the thin-walled paired kidneys, which lie on either side of the gut posterior to the ventricle. Because of the tenuous nature of the epithelia, the paired anterior connection with the pericardial cavity was not discovered with complete certainty, but it was thought to originate close to the vessel leading to the mantle sinus. The ducts to the exterior pass to the dorsal side of the body, below the dorsal mantle sinus.

The gross morphology of the heart and kidney of *Nucinella serrei* corresponds very closely with that described by Stempell (1899) for *Solemya togata*. In contrast with the gut, the heart is very large in *Nucinella*, in fact for such a small animal the blood system as a whole seems disproportionately large. In this way *Nucinella* resembles *Solemya* (Owen, 1961).

DISCUSSION

The foot, blood system, digestive system, gills and palps all emphasize the close affinity of *Nucinella serrei* to the Solemyidae. Hinge characters, monomyarian condition and lack of mantle fusion all show that despite this affinity, the Nucinellidae are a distinct group. Although the Nucinellidae exhibit great internal differences and external shell characters (particularly shape), they recall the Nuculidae. In other words, there has been less change in the orientation of the body axis than in *Solemya*

(Figs. 3 and 5). The single adductor muscle remains anterior in position, and the lack of the posterior adductor is compensated for by the long anterior lateral hinge tooth, the posterior position of the external ligament and the disposition of the posterior pedal retractors. In other monomyarians, e.g., *Pecten*, the posterior adductor muscle tends to become centrally placed while the anterior adductor is lost. It should also be noted that in the development of any dimarian bivalve the anterior adductor is formed before the posterior. The condition in *Nucinella* resembles this, although it is debatable whether this is an example of neotony.

The evolutionary history of the Protobranchia is not easily determined. Among the earliest fossil bivalves are the Ctenondonata found in the Upper Cambrian (Cox, 1959). The known species of the Ctenodonta possess a row of transversely set taxodont teeth, which, in some species, are chevron shaped. These species have no resilifer and are possibly the ancestral stock of the present day Malletiidae. They do not resemble *Nucinella*.

Most Ordovician, non-protobranch bivalves have been placed by different workers at various times within the order Actinodonta. Pojeta (in press) points out the heterogeneous nature of this grouping and restricts the term 'actinodont' to the families Cycloconchidae, Lyrodesmatidae, Redoniidae, and such non-Ordovician families as the Caryiidae. He defines these forms as equivalved, non-byssate, inequilateral bivalves with an opisthodetic ligament. The dentition was often ventrally divergent, with all the teeth reaching or nearly reaching the beaks[5]. In the more primitive members, the posterior teeth extended under the ligament the full length of the dorsal margin posterior to the beaks. All members were dimyarian with subequal abductors, with the anterior adductor supported by a myophoric buttress on its posterior margin, and with two or more pairs of pedal muscles.

Nucinella, by being equivalved, non-byssate, inequilateral, possessing an opisthodetic ligament, ventrally divergent dentition with all the teeth nearly reaching the beaks, an anterior adductor muscle supported by a myophoric buttress on its posterior margin, and with three pairs of pedal muscles, shares a number of the actinodont features. It departs from the actinodont morphology by having only a single adductor muscle, and lacking or having a much reduced posterior lateral tooth. Functionally, however, the absence of the posterior adductor muscle and the posterior lateral teeth can be related to the pronounced truncation of the body posterior to the umbo, although here it is difficult to determine what is cause and what is effect.

Even though *Nucinella* does not precisely satisfy the above definition of an actinodont, it does, in our opinion, fit this order better than any of the remaining bivalve groups. Perhaps it should be considered as a rather specialized member of the Actinodonta. Yet, information presented in the present paper amply demonstrates that members of the family Nucinellidae are protobranchs whose closest living relatives are the Solemyidae. If *Nucinella* is an actinodont, can we not conclude that the actinodonts and the protobranchs together represent but a single order of bivalves?

Assuming that *Nucinella* can be derived from the actinodonts and has, therefore, most ancient lineage, can its anatomy give any clue to the possible anatomy of earliest bivalve? Most significant perhaps is that the gills are large and the palps small. It has always

[5]A number of abyssal protobranch species have been obtained recently that have a few elongate lateral non-taxodont teeth arranged parallel to the hinge line. From consideration of other characters, these species are related to the Nuculanidae and the Malletiidae. We will describe these in a future publication.

seemed somewhat paradoxical that the Nuculidae, considered to be among the most primitive bivalves, have such a highly specialized palp feeding mechanism, a complex stomach and relatively small gills. It is suggested that *Nucinella* with its simple palps and simple stomach, possible reflects more the condition of the early bivalve. Food is not sorted in the stomach of *Nucinella* and there seems to be no reason why this should not be the primary condition in bivalves.

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RÉSUMÉ

NUCINELLA SERREI (BIVALVIA: PROTOBRANCHIA), UN SOLEMYIDAE MONOMYAIRE ET POSSIBLE ACTINODONTE VIVANT

J. A. Allen et H. L. Sanders

Des exemplaires de *Nucinella serrei* Lamy obtenus au large de la Caroline du Nord, U. S. A., (Lat. 34° 16,6' N: Long. 75° 68,6' W) à une profondeur de 450 m, ont permis de donner la première description morphologique des parties molles d'un représentant de la famille des Nucinellidae.

Nucinella serrei est unique, en ce sens qu'il est le premier bivalve monomyaire décrit chez lequel le muscle adducteur manquant est le postérieur et non l'antérieur. Qui plus est cet unique muscle adducteur devenu plus fort, demeure bien en position antérieure. L'absence de muscle adducteur postérieur est compensé par la position postérieure extrême du ligament opisthodète, par le grand développement des muscles postérieurs rétracteurs du pied et par la longue dent latérale antérieure. Cette dernière empêche l'écartement sagittal des 2 valves. Le ligament et la rétracteur du pied assurent l'attachement postérieur des 2 valves.

L'analyse de sa morphologie montre que *Nucinella serrei* est un bivalve protobranche et qu'il est étroitement apparenté aux Solemyidae. Les branchies sont très semblables à celles de *Solemya* et de très petits palpes non striés sont présents. Le pied est volumineux et a une sole divisée en deux longitudinalement et bordée de grosses papilles. Il n'est pas dirigé vers l'avant comme chez *Solemya*, mais il se tient dans la même position et a la même allure que le pied de *Nucula*. Il y a une paire de statocystes bien développés, situés dorsalement et latéralement par rapport au ganglion pédieux.

L'intestin de *Nucinella serrei* est simple et ressemble à celui de *Solemya*. L'estomac est intérieurement recouvert de chitine sauf dans un étroit sillon ventral, qui conduit, au moyen d'une simple ouverture, à un diverticule digestif. Le sillon se prolonge, postérieurement à l'ouverture, comme voie unique de rejection conduisant à l'intestin moyen. Le court sac du stylet cristallin est en relation avec l'intestin moyen. Les diverticules digestifs s'ouvrent à droite et à gauche d'un simple "vestibule" qui est en position antéro-ventrale par rapport à l'ouverture de l'estomac. De chaque côté les diverticules sont divisés antérieurement en un saccule et postérieurement en 4 tubules digités, chacun ayant une lumière très étroite.

Le coeur de *Nucinella serrei* est volumineux et le système circulatoire très développé. Un ensemble de sinus sanguins occupe une grande partie de la cavité générale du corps.

On suggère qu'il est possible que *Nucinella serrei* puisse dériver des Bivalves actinodontes de l'Ordovicien inférieur et que l'intestin simple, les palpes réduits et les épaisses branchies représentent des caractères primitifs de bivalves (protobranches) plus que l'intestin complexe et les palpes des Nucules actuelles.

RESUMEN

NUCINELLA SERREI (BIVALVIA: PROTOBRANCHIA),
UN MONOMIARIO SOLEMIDO Y POSIBLE PREPRESENTANTE
VIVIENTE DE LOS BIVALVOS ACTINODONTES

J. A. Allen y H. L. Sanders

Ejemplares de *Nucinella serrei* Lamy, obtenidos frente a Carolina del Norte (lat. 34 16'16'' N, long. 68. 6' W) a 450 metros de profundidad, han permitido la primera descripción de la morfología interna de un componente de la familia Nucinellidae.

La singularidad de *Nucinella serrei* es la de ser el primer monomiario en el cual, el músculo aductor posterior, y no el anterior, es el ausente. Además, el aductoanterior único, agrandado, permanece en posición anterior. La falta del aductor posterior se compensa por la posición posterior externa del ligamento opistodetico, los músculos retractores pedales bien desarrollados y el largo diente lateralanterior; este último previene que las valvas se desvien sagitalmente.

El análisis morfológico mostró que *Nucinella serrei* es un protobranquio, y que se relaciona estrechamente con los Solemyidae. Las branquias son muy similares a aquellas de *Solemyia* y tiene palpos lisos muy pequeños. El pié, grande, tiene la suela dividida longitudinalmente, la que está floqueada por papilas grandes; no está dirigido hacia adelante como en *Solemyia*, sino que se mantiene en la misma posición y actitud del pié de *Nucula*. Hay un par de estatocistos bien desarrollados, dorsales y laterales al ganglio pedal.

El canal alimenticio es simple y recuerda al de *Solemyia*. El estómago esta forrado de quitina excepto en un estrecho surco ventral que conduce, por medio de una abertura simple, a los divertículos intestinales. Este surco continua, posteriormente a la abertura, como el único tracto de desecho que conduce al intestino medio. Los divertículos intestinales se abren a la derecha e izquierda de un "vestíbulo" simple, colocado antero-ventralmente a la abertura, desde el estómago. A cada lado, los divertículos se dividen anteriormente en una posición sacular posterior, en 4 tubos digitos, cada uno con un lumen muy estrecho.

El corazón de *Nucinella serrei* es grande, y el sistema circulatorio muy extenso. Un complejo de senos hemolinfaticos ocupa gran parte de la cavidad del cuerpo.

Se sugiere la posibilidad de que *Nucinella serrei* derive de los bivalvos actinodontes del Ordovicico inferior, y que el intestino simple, pequeños palpos, y branquias grandes, representan la condicion primitiva de los (protobranquios) bivalvos, en lugar del complejo intestino y palpos de los nuculidos recientes.

АБСТРАКТ

NUCINELLA SERREI (BIVALVIA, PROTOBRANCHIA),
ОДНОМУСКУЛЬНАЯ СОЛЕМИИДА И, ВОЗМОЖНО, НЫНЕ ЖИВУЩАЯ
АКТИНОДОНТА

ДЖ. А. АЛЛИН И Г. Л. САНДЕРС

На экземплярах *Nucinella serrei* Lamy, найденных близ берегов Северной Каролины, США, (34°16,6' сев.ш., 75°68,6' зап.д на глубине 450 м впервые для представителя семейства Nucinellidae было сделано морфологическое описание тела.

Nucinella serrei является уникальной в том смысле, что является впервые описываемым представителем мономиарных двустворчатых, у которых отсутствует задний (а не передний) муску замыкатель. Кроме того, этот увеличенный в размере единственный передний аддуктор, остается по своему местоположению передним. Отсутствие заднего мускула-аддуктора компенсируется крайним задним расположением опистодонтного лигамента, хорошо развитыми задними ножными мускулами-ретракторами и длинным

передним боковым зубом. Последний, предотвращает сагиттальное смещение обеих створок, а лигамент и ножные ретракторы обеспечивают соединение обеих створок сзади.

Анализ морфологии тела моллюска показывает, что ***Nucinella serrei*** представляет собою двустворчатого моллюска из протобранхий и что он близко-родственен **Solemyidae**. Жабры очень сходны с жабрами ***Solemya;*** имеются очень маленькие неборозлчатые пальпы. Крупная нога имеет продольно-складывающуюся подошву с крупными папиллами по краям. Нога не направлена вперед, как у ***Solemya***, но находится в том же положении и направлении, как нога у ***Nucula***. Имеется пара хорошо развитых статоцистов, расположенных дорзально и латерально по отношению к ножному ганглию.

Кишка у ***Nucinella serrei*** - простая и сходна с кишкой у ***Solemya***. Желудок выстлан хитином, кроме узкого желобка с брюшной стороны, который открывается единственным отверстием в пищеварительную дивертикулу. Желобок продолжается назад вплоть до отверстия, служа единственным выводным трактом, ведущим в среднюю кишку. Короткий мешок стебелька **(style sack)** связан со средней кишкой. Пищеварительная дивертикула открывается направо и налево от единой камеры, расположенной антеро-вентрально по отношению к отверстию из желудка. С каждой стороны дивертикула разделена спереди на мешковидную часть, а сзади - на 4 пальцевидных трубочки, каждая с очень узким просветом.

Сердце у ***Nucinella serrei*** крупное и кровеносная система очень разветвленная. Комплекс кровеносных синусов занимает большую часть полости тела.

авторы предполагают, что весьма возможно, что ***Nucinella serrei*** может происходить от актинодонтных двустворчатых Нижнего Ордовика и что просто устроенная кишка, маленькие пальпы и крупные жабры служат признаками большей примитивности у двустворчатых протобанхий, чем сложные кишка и пальпы современных нукулид.

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THE MUSSEL RESOURCE OF THE TENNESSEE RIVER

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ABSTRACT

This study is an effort to assess the status of the mussel fauna in the Tennessee River as of 1965 and to compare it to the rich endemic fauna present prior to impoundment by the Tennessee Valley Authority in 1936. Many species have been important to the pearl button industry in the past and some are now important to the pearl culture industry.

Yearly harvests of about 10,000 tons of shells in the 1940's and 1950's have steadily declined, falling to 2,000 tons from 1964 through 1967. Mussel populations have been significantly affected by impoundment in species composition and in distribution. While impoundment has been the primary cause of decline by reducing suitable mussel habitats, overharvesting of mussels in the river-like portions below the dams (tailwaters) has recently resulted in a further rapid depletion of the mussel resource.

Approximately 175 miles of suitable habitat remain in the first 531 miles of river, mostly in the tailwaters. The present fauna comprises 44 species as against about 100 formerly recorded. The drastic reduction of "Cumberlandian" species (6 against 25) was confirmed. Many of the species still present have become rare. Several species have only recently become established in their present locations.

Legislation regulating mussel harvesting, enacted in 1965-1966, should help halt the rapid depletion of mussel resources. The range of silt-tolerant species is gradually extending, but before the commercially important mussels, in particular *Fusconaia ebenus* and *Pleurobema cordatum* can be successfully managed, their life histories will have to be elucidated. The future of the mussel industry remains uncertain at the present time.

CONTENTS

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INTRODUCTION

Judging from the size and number of pre-Columbian shell middens still visible along the Tennessee River, freshwater mollusks have long been valued by man. Apparently, in those early days, naiads or freshwater mussels were used mainly for food. Some shell material was utilized in burials, some were fashioned into drinking cups or instruments for digging or hoeing. Mussel shells were also cut, rounded and strung as beads, along with gastropod shells. Archeological records indicate a general absence of pearls in Tennessee Valley area burial grounds.

With the advent of the pearl button industry on the Mississippi River (Muscatine, Iowa) in the latter part of the 19th century, the Tennessee River became a source of raw material for pearl buttons. In 1883 a short-lived freshwater pearl button plant was established at Knoxville, Tennessee, which was soon discontinued for lack of suitable machinery. In the early 20th century the Tennessee Valley mussel resources were increasingly utilized by the American button industry. Coker (1919) valued the 1912 Tennessee River mussel harvest at $11,000 and a similar value was given to the 1914 harvest of 650 tons in an early Bureau of Fisheries report. The yearly tonnage and total values generally increased in subsequent years.

In 1936, when the Tennessee Valley Authority (TVA) completed Wheeler Dam, the first mainstream dam constructed by TVA (Wilson Dam was constructed by the government earlier), near Elgin, Alabama, (Fig. 1; Table 1), mussel fishermen and some, but not all, biologists in the TVA and the U.S. Bureau of Fisheries predicted that planned impoundments on the Tennessee River would eliminate most of the suitable mussel habitats. Mussel fishermen stopped harvesting on the Wheeler Reservoir portion of the Tennessee River as soon as the reservoir filled.

Nine years later musselers made exploratory drag samples in Wheeler Reservoir and found large beds of mussels. As a result, mussel fishing was resumed throughout the river and the shell harvest on the Tennessee River increased dramatically from 3,700 tons in 1945 to over 10,000 tons in 1947. Earlier forecasts that impoundment would be detrimental to freshwater mussels was soon forgotten.

Among the more important and abundant mussel species that have been commercially exploited are the following: *Pleurobema cordatum* (Rafinesque), *Fusconaia ebenus* (Lea), *Amblema costata* (Rafinesque), *Megalonaias gigantea* (Barnes), *Quadrula quadrula* (Rafinesque), *Q. pustulosa* (Lea), *Q. metanevra* (Rafinesque), *Plagiola lineolata* (Rafinesque), *Ligumia recta latissima* (Lamarck) and *Lampsilis anodontoides* (Lea) (Table 2); some other species with shells of less desirable quality were also harvested. Button industry demand for shells continued through the years but at a declining rate, as plastics and other materials entered the market. However in the mid-1950's a lucrative export market for Tennessee River shells developed with demands from the Japanese cultured pearl industry. Rounded pieces of shell are inserted into live "oysters" or into freshwater mussels which cover the shell nuclei with a veneer of pearl. The resulting cultured pearls are the basis of an estimated $85-million-a-year Japanese business. With this stimulus, mussel fishing again accelerated. However, annual mussel harvests, which for years had topped the 10,000 ton level, declined from 1956 onwards, despite increased efforts in mussel fishing. In 1956-1957 the U.S. Fish and Wildlife service undertook a study of mussel beds in the Wheeler Reservoir portion of the Tennessee River and indicated unfavorable environmental changes since impoundment as the primary factor affecting natural recruitment.

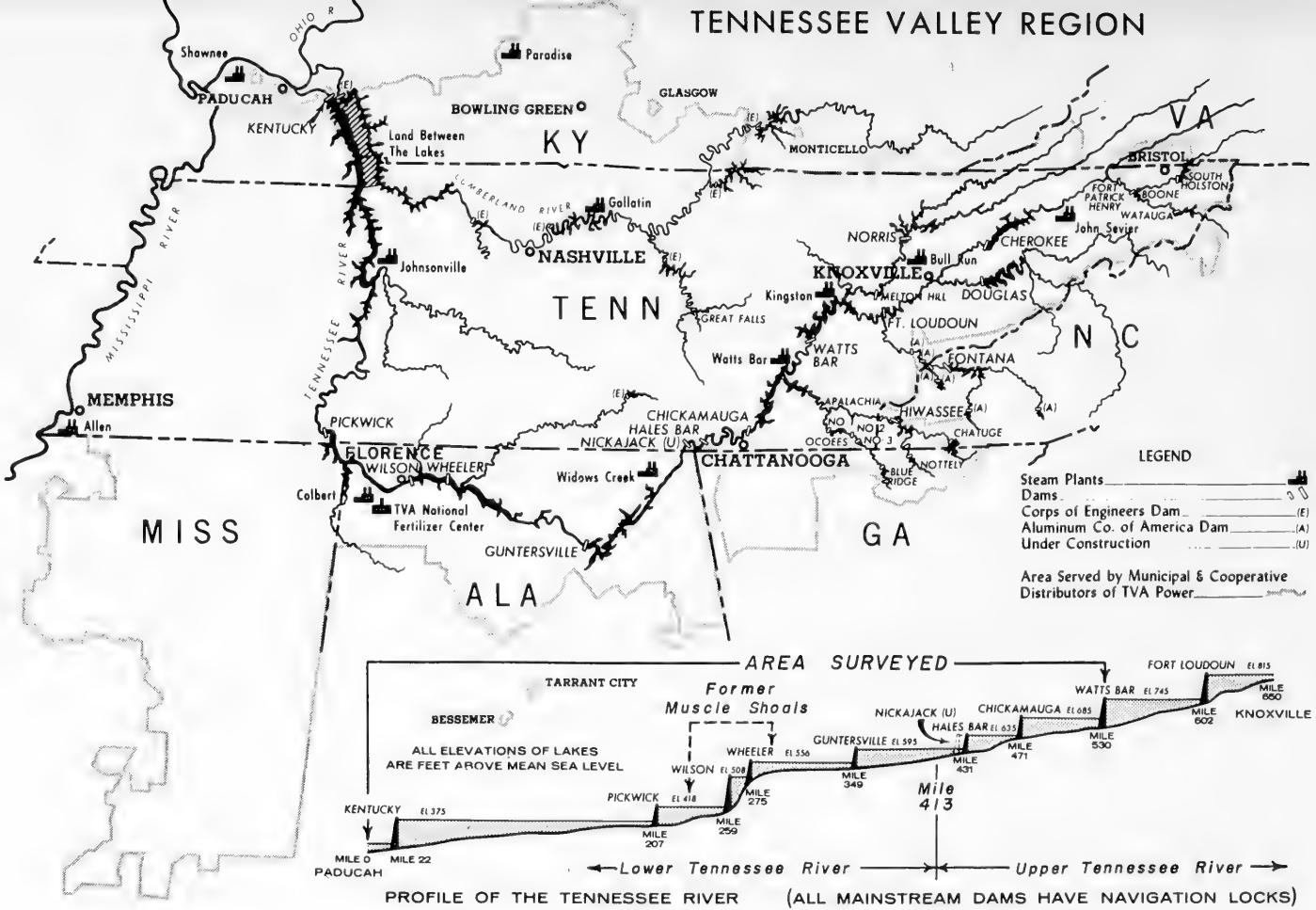

FIG. 1. Map of the Tennessee and Cumberland River basins showing impoundments, 1965. Abbreviations: ALA = Alabama; GA = Georgia; KY = Kentucky; MISS = Mississippi; NC = North Carolina; TENN = Tennessee; TVA = Tennessee Valley Authority; VA = Virginia.

In July 1963, the Tennessee Valley Authority began an extensive investigation to determine the reasons for the continued drop in mussel harvest. The record showed that annual harvest declined 53% between 1960 and 1962 and catch-per-boat data suggest a downward trend probably starting as early as 1954.

Lending support to and participating in the study were the U.S. Fish and Wildlife Service, the Alabama Department of Conservation, the Kentucky Department of Fish and Wildlife Resources, the Tennessee Game and Fish Commission, and some of the major shell companies. Water quality data were furnished by TVA's Health and Safety Division. The Division of Water Control Planning supplied geological information and data on sediment accumulation.

Specific goals of the study were to determine the location, distribution and density of the mussel populations, suitability of habitats, presence of disease, extent of parasitism, to begin an investigation of the life cycle of some commercial mussel species, and to define the needs for conservation. It was hoped that findings might lead to ways and means of sustaining an annual shell harvest at a level of 5,000 - 9,000 tons.

The Tennessee River was examined from its mouth, at its confluence with the Ohio River, upstream to Watts Bar Dam, a distance of 529 miles (See profile, Fig. 1). No concentrations of commercially important mussels were known above that dam. Location of mussel "beds" was determined by systematic sampling and by observation of mussel harvesters. Quantitative sampling of beds supplied data on species composition and distribution. Water quality and habitat conditions were especially noted.

SHELL HARVEST

As already stated, Tennessee Valley mussel resources were heavily used by the American pearl button industry from about 1900-1955, when washable plastics became the industry's principal raw material. Since then, Tennessee River shells have been used primarily by the Japanese cultured pearl industry.

While the yield in tons was measured in the hundreds in the early years, mussel harvest averaged 9,600 tons from 1945 through 1955, but fell from 11,500 tons in 1955 to 6,600 tons in 1956 (see Table 3). Following several years of low harvest, higher prices and greater effort again boosted the catch to 10,000 tons in 1960. But it then fell to 7,000 tons in 1961 and to 4,700 tons in 1962. This continued decline occurred even though more mussel fishing boats operated, with more efficient equipment, on the Alabama and Tennessee reservoirs than ever before (Fig. 2). The value of the harvest similarly increased with the years. Total value from 1945 through 1967 was in excess of $11 million, with an annual peak of $1.2 million in 1960 (Table 3). As many as 1,000 people have been involved in a single annual harvest. Between 1954 and 1963 the average price of shells increased from $42 to $147 per ton, and was accompanied by a threefold increase in the number of mussel fishing boats; but, despite the increased pressure, total shell harvest dropped from 11,000 to 5,800 tons a year. Subsequently, with an effort comparable to that made at the time of maximum yield, the annual harvest was stabilized at somewhat over 2,000 tons a year.

HISTORICAL REVIEW OF MUSSEL FAUNA

The great variety of mussel species formerly occurring in the Tennessee and Cumberland river basins has prompted a number of studies of this characteristic, endemic, fauna. It comprises species thought to have originated in the upper Cumberland and upper Tennessee river basins

TABLE 1. Tennessee River Dams

Name of Dam	Location (State)	Ascending River Mile	Date of Closure
Kentucky	Kentucky	22.4	August, 1944
Pickwick	Tennessee	206.7	February, 1938
Wilson*	Alabama	259.4	April, 1924
Wheeler	Alabama	274.9	October, 1936
Guntersville	Alabama	349.0	January, 1939
Nickajack	Tennessee	424.7	November, 1967
Hales Bar**	Tennessee	431.1	October, 1913
Chickamauga	Tennessee	471.0	January, 1940
Watts Bar	Tennessee	529.9	January, 1942
Fort Loudoun	Tennessee	602.3	August, 1943

*Was acquired from the U.S. Corps of Engineers in 1933.
**Was replaced by Nickajack Dam. Hales Bar was purchased from Tennessee Electric Power Company in 1939.

TABLE 3. Annual Shell Harvest, Tennessee River 1945 - 1967

Year	Number of boats (approx.)	Average tons per boat	Average value* per ton $	Total shells (tons)	Total value* $
1945	143	26.01	40	3,720	148,660
1946	149	66.28	38	9,875	373,781
1947	186	57.04	39	10,610	410,540
1948	210	55.54	43	11,663	502,229
1949	200	37.85	35	7,570	265,000
1950	228	32.01	30	10,500	315,000
1951	256	40.00	40	10,241	409,640
1952	256	31.73	45	8,124	365,580
1953	261	41.72	55	10,890	600,518
1954	280	40.07	42	11,220	472,975
1955	298	38.47	44	11,463	504,252
1956	280	23.58	59	6,603	390,583
1957	317	23.27	75	7,376	556,026
1958	294	16.33	60	4,802	288,120
1959	519	10.80	69	5,606	389,616
1960	861	12.06	122	10,380	1,267,875
1961	926	7.60	125	7,039	882,397
1962	802	5.59	141	4,716**	666,548
1963	678	8.10	147	5,800***	852,911
1964	398	5.30	139	2,112	294,385
1965	233	10.37	143	2,418	346,121
1966	268	10.20	211	2,734	577,161
1967	366	6.45	182	2,361	428,561

*Based on river bank prices.
**Divers collected 235 tons.
***Divers collected 212 tons, dredge boats 97 tons.

TABLE 2. Naiads inhabiting the Tennessee River (TRM 0-529), 1965

Species	Common Name*	Faunal Group	Commercial Importance
Margaritiferidae			
Cumberlandia monodonta (Say, 1829)	Spectacle-case	Unknown	-
Unionidae			
Unioninae			
Fusconaia flava f. *undata* (Barnes, 1823)	Pigtoe	--	-
Fusconaia ebenus (Lea, 1831)	Black Mussel**	Ohioan	+++
Fusconaia subrotunda (Lea, 1831)	Long solid	--	+
Megalonaias gigantea (Barnes, 1823)	Washboard	Ohioan	++
Amblema costata (Refinesque, 1820)	Three-ridge	--	++
Quadrula quadrula (Rafinesque, 1820)	Maple-leaf	Ohioan	++
Quadrula pustulosa (Lea, 1831)	White wartyback	--	+
Quadrula metanevra (Rafinesque, 1820)	Monkeyface	Ohioan	+
Quadrula cylindrica (Say, 1817)	Rabbits-foot	--	-
Tritogonia verrucosa (Rafinesque, 1820)	Pistolgrip	--	-
Cyclonaias tuberculata granifera (Lea, 1838)	Pink wartyback	--	-
Plethobasus cyphyus(Rafinesque, 1820)	Bullhead	--	-
Plethobasus cicatricosus (Say, 1829)	White wartyback	Ohioan	-
Plethobasus cooperianus (Lea, 1834)	Pimpleback	Ohioan	-
Lexingtonia dolabelloides (Lea, 1840)	none	Cumberlandian	-
Pleurobema oviforme (Conrad, 1834)	none	Cumberlandian	-
Pleurobema cordatum (Rafinesque, 1820)	Ohio River Pigtoe	--	+++
Elliptio crassidens (Lamarck, 1819)	Elephant's-ear	--	-
Elliptio dilatatus (Rafinesque, 1820)	Ladyfinger	--	-
Anodontinae			
Arcidens confragosus (Say, 1829)	Rock Pocketbook	--	-
Lasmigona complanata (Barnes, 1823)	White heelsplitter	Ohioan	-
Anodonta grandis (Say, 1829)	Floater	Ohioan	-
Anodonta corpulenta (Cooper, 1834)	slop-bucket	--	-
Anodonta imbecillis (Say, 1829)	Floater or Paper-shell	--	-
Anodonta suborbiculata (Say, 1831)	Paper-shell	Ohioan	-

Table 2 (contd.)

Species	Common Name*	Faunal Group	Commercial Importance
Lampsilinae			
Ptychobranchus fasciolaris (Rafinesque, 1820)	Kidney-shell	--	-
Obliquaria reflexa (Rafinesque, 1820)	Three-horn	--	-
Cyprogenia irrorata (Lea, 1829)	Goat's-foot or Fan-shell	--	-
Obovaria olivaria (Rafinesque, 1820)	Egg-shell or Hickory-nut	Ohioan	-
Obovaria retusa (Lamarck, 1819)	Golf-stick or Ring-pink	Ohioan	-
Actinonaias carinata (Simpson, 1900)	Mucket or Southern Mucket	--	-
Plagiola lineolata (Rafinesque, 1820)	Butterfly	Ohioan	+
Truncilla truncata (Rafinesque, 1820)	Deertoe	Ohioan	-
Truncilla donaciformis (Lea, 1829)	Deertoe or Fawn's-foot	Ohioan	-
Leptodea laevissima (Lea, 1829)***	Paper-shell	--	-
Leptodea fragilis (Rafinesque, 1820)	Fragile-paper	--	-
Proptera alata (Say, 1817)	Heelsplitter	--	-
Proptera laevissima (Lea, 1829)***	Paper-shell	Ohioan	-
Carunculina parva (Barnes, 1823)	Lilliput	--	-
Ligumia recta latissima (Lamarck, 1819)	Black sandshell	--	+
Lampsilis anodontoides (Lea, 1831)	Yellow sandshell	Ohioan	+
Lampsilis ovata (Say, 1817)	Pocketbook or Grandma	--	-
Lampsilis orbiculata (Hildreth, 1828)	Pink mucket	Ohioan	-

*Common names are mostly after Coker, 1915.

**It is proposed that Black Mussel be substituted for "Niggerhead" (Coker, 1915) as the common name for this species.

***These 2 species are synonymous

-- = of unknown or doubtful origin

+++, ++, + - = degree of importance in descending order.

TRM = Tennessee River mile (ascending)

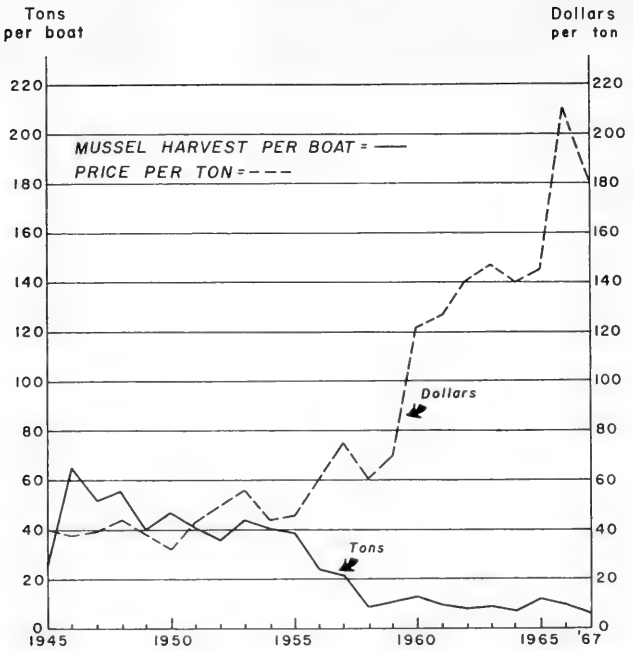

FIG. 2. Annual mussel shell harvest in the Tennessee River, 1945 - 1967.

TABLE 4. Sizes of *Fusconaia ebenus* collected in Kentucky Reservoir and Kentucky and Pickwick Dam Tailwaters, Tennessee River, 1964

Number growth lines	Number of specimens	Average measurements in mm		
		length	height	width
3	26	35.6	32.3	22.4
4	77	41.0	36.5	25.1
5	42	46.8	41.9	28.4
6	18	53.0	45.9	32.2
7	18	58.3	51.3	36.0
8	6	66.9	56.7	40.2
9	1	79.0	73.0	45.0
10	6	69.4	61.1	42.3
11	4	62.5	56.2	39.7
12	3	77.5	65.9	45.6
13	1	83.2	67.8	51.1
14	2	84.4	75.4	50.2
16	1	84.5	71.9	49.3
22	1	92.6	75.0	50.0
Total	206			
Range		23.6-86.0	21.0-84.0	14.6-48.0
Mean		47.9	42.4	29.1
Standard deviation		13.7	11.7	7.9
Standard error		0.96	0.81	9.55

(Cumberlandian fauna) and in the "Interior Basin" (Ohioan fauna), along with species of undertermined origin.

Ortmann (1925) and van der Schalie (1939) have provided basic information on mussels of the lower Tennessee River. Ortmann's paper was based on comprehensive collections from 4 stations below Walden Gorge (between Bridgeport, Alabama, TRM[2] 413.0, and Paducah, Kentucky TRM 0) and on previous records. His (1925) study, together with an earlier one (1918) on the naiads of the upper Tennessee drainage (above Walden Gorge, including upstream tributaries) are the basic guides to the Tennessee River Mussel fauna. The van der Schalie report was based largely on collections from the Tennessee River made by M. M. Ellis in 1931. Approximately 100 species, including 25 Cumberlandian forms, have been recorded in the literature from this area.

Van der Schalie was particularly concerned about the effects of impoundment on mussels in the lower Tennessee River: "The Tennessee Valley Authority undoubtedly will alter completely the ecological conditions for the naiads inhabiting the lower Tennessee River. If, after the impounding of waters, the reaction of the mussels now occupying that region is similar to that of mussels found in impounded areas elsewhere, we can predict safely that the proposed Gilbertsville Dam at Paducah will entirely change the fauna now found in the lower Tennessee." Kentucky Dam was eventually completed on this site in 1944.

Ortmann (1925) defines the "Interior Basin" naiad fauna as comprising those species that had "their center of disperal in the lower Ohio and its vicinity." Van der Schalie (1939) considered 25 species as "belonging to the naiad fauna of the Interior Basin of the Lower Tennessee." However, he only listed 24 species and his No. 19 was not included in the check list. Probably it is *Plagiola lineolata*, an Interior Basin species belonging to the lower Tennessee River. Van der Schalie's species list constitutes the final historical record for the pre-impoundment "Ohioan" fauna of the lower Tennessee River, since, with the progressive restriction of the endemic mussel fauna to stretches below the dams, further contributions as to the original fauna and distribution have become impossible.

Three recent studies reveal some of the changes that have since taken place in the lower Tennessee River. Scruggs (1960) treated the status of freshwater mussel stocks in the river; Bates (1962) dealt with the effects of impoundment on the mussel fauna of Kentucky Reservoir, especially that of postimpoundment mud-sand shallows, and Stansbery (1964) discussed the changes in the fauna of the famous "Mussel (Muscle) Shoals" immediately below Wilson Dam.

Bates (1962) noted that, of the Unioninae present in the area before impoundment, the only species that had successfully invaded the new "virgin" shallows of Kentucky Reservoir by 1958 was *Quadrula quadrula*, a species which Ortmann (1925) had already identified as a "mud-lover".

Regarding the Anodontinae, van der Schalie (1939) had reported them as being completely absent from the lower Tennessee River. However, Bates (1962) recorded 3 species of *Anodonta* from Kentucky Reservoir in Tennessee.

CAUSES OF MUSSEL DECLINE

In the assessment and survey various possible avenues were explored that would explain the reasons for the observed decline of commercially important mussels. Dissection and microscopic examination of over 140 specimens representing the mussel population

[2]TRM = Tennessee River Mile, counted from the mouth of the river.

TABLE 5. Sizes of *Pleurobema cordatum* collected in Kentucky Reservoir and Kentucky and Pickwick Dam Tailwaters, Tennessee River, 1964

Number growth lines	Number of specimens	Average measurements in mm		
		length	height	width
3	1	38.5	38.4	23.3
4	8	42.6	38.9	25.5
5	4	48.0	44.1	29.2
6	8	58.4	51.9	34.0
7	3	66.1	62.2	39.1
8	3	70.7	62.3	40.3
9	3	71.6	66.4	43.8
10	3	68.8	66.7	42.6
11	2	69.0	60.0	41.3
12	3	81.9	74.4	47.0
13	1	88.0	73.0	42.0
14	1	83.0	69.0	50.0
15	1	77.0	67.8	47.2
16	3	86.0	72.7	49.3
18	1	93.0	76.0	51.0
19	1	78.2	67.8	47.4
21	1	75.0	72.0	51.0
24	1	80.2	64.7	48.6
Total	48			
Range		36.7-93.0	30.6-76.0	20.6-51.0
Mean		64.3	57.6	38.0
Standard deviation		15.8	13.2	8.9
Standard error		2.3	1.9	1.3

TABLE 7. Relative abundance of dominant mussel species from beds in (I) Kentucky Dam Tailwater, (II) Kentucky Reservoir, and (III) Guntersville Dam Tailwater (1964)

Species	Faunal origin	I	II	III
			Abundance*	
Fusconaia ebenus	Ohioan	1	1	**
Pleurobema cordatum	--	2	2	2
Quadrula pustulosa	--	3	4	3
Amblema costata	--	4	-	5
Obliquaria reflexa	--	5	3	1
Quadrula quadrula	Ohioan	6	-	***
Plagiola lineolata	Ohioan	7	5	7
Elliptio crassidens	--	-	6	6
Megalonaias gigantea	Ohioan	-	6	-
Cyclonaias tuberculata granifera	--	-	7	4

*Rank based on greatest density per square yard in descending order.

**Not obtained in collection from this tailwater; however, Scruggs (1960) reported finding *Fusconaia ebenus* in both Guntersville and Chickamauga reservoirs which are upstream from this station.

***Obtained in 1963 but not in 1964.

-- = unknown.

TABLE 6. Mussel Density in Kentucky Dam Tailwater and Reservoir, Lower Tennessee River, 1964

Species	Estimated number per square yard	
	Tailwater beds	Reservoir beds
Fusconaia flava f. *undata*	*	-
Fusconaia ebenus (incl. *subrotunda*)	1.02	0.849
Megalonaias gigantea	0.11	0.047
Amblema costata	0.18	0.011
Quadrula quadrula	0.13	0.016
Quadrula pustulosa	0.31	0.143
Quadrula metanevra	*	0.022
Quadrula cylindrica	*	-
Tritogonia verrucosa	*	0.007
Cyclonaias tuberculata granifera	0.03	0.045
Pleurobema cordatum (incl. *pyramidatum*)	0.37	0.234
Elliptio crassidens	0.02	0.047
Elliptio dilatatus	0.07	-
Anodonta imbecillis	-	0.002
Obliquaria reflexa	0.14	0.192
Cyprogenia irrorata	*	0.029
Plagiola lineolata	0.12	0.074
Truncilla donaciformis	0.01	0.005
Proptera alata	0.01	0.007
Ligumia recta latissima	**	0.007
Lampsilis anodontoides	**	0.002
Lampsilis orbiculata	-	0.018
Lampsilis ovata	-	0.009

*Present, but not taken in quantitative samples.
**Taken by qualitative brail sampling only.

immediately downstream from Guntersville Dam (see Table 8) and gross examination of scores more from other areas did not reveal the presence of disease or extensive parasitism. Larval trematodes and water mites were the most common parasites noted. The latter were most numerous in *Obliquaria reflexa*. The valuable *Pleurobema cordatum* was virtually free of parasites.

Industrial pollution has been blamed for mussel yield decline, but there is little evidence to indicate that it has been a serious factor. Water quality is less than desirable in some isolated areas, but the general decline in yield below the dams cannot be blamed on pollution. Studies by the TVA Health and Safety Division, in Watts Bar, Chickamauga, Guntersville, Wheeler, Wilson and Pickwick Reservoirs (Staff Reports 1964a,b) show generally good water quality, and this is also true in Kentucky Reservoir.

One other possible contribution to mussel decline may be the substantial intrusion of Asiatic clams (*Corbicula manillensis* Philippi) into all reservoir habitats: densities were noted to approximate 2,000 per square meter in some areas.

Overharvesting is certainly involved, especially in the river-like reaches immediately below the dams. According to Scruggs (1960) the 1956-1957 yield of commercial size (65mm) *Pleurobema cordatum* on the Triana mussel bed (Wheeler Reservoir) was 23 times natural replacement. About 90% of specimens were older than 12 years. Annual yield in the entire valley, which had already fallen by over 50% between 1960 and 1962, declined by another 64% in 1964. The continued low yields in the years since 1964 (Table 3) suggest overharvesting. It can be added that yields are low despite acceptance of shells formerly thought undesirable.

Sediment has affected much of the aquatic habitat in the reservoirs and to some extent in the areas immediately below the dams. The most notable effect on the latter has been filling of the interstices (impaction) of the gravel beds with sediment. Quantitative data on sedimentation provided by the TVA Hydraulic Data Branch, together with the sampling done in the present study, reveal the profound changes that have taken place in the former mussel habitats due to impoundment.

Commercially important mussel species do better on a clean gravel or firm mud bottom and will not tolerate the thin film of sediment that now covers much of the bottom of the old river channel. Ellis (1931) pointed out that "The Tennessee River above Paducah, Kentucky, was observed to change from a relatively clear stream to one turbid with silt in suspension in the course of a few hours following a local thunderstorm in the Duck River region. . . The deposition of erosion silt behind any obstruction to streamflow, be it temporary or permanent, covers all mussel beds above this obstruction for a distance depending upon the size of obstruction." He also reported that erosion silt covers young mussels and decreases their survival rate in areas where adult mussels may be present.

Impoundment has reduced the current in the Tennessee River to the extent that it no longer keeps the substrate swept clean. This is why the largest mussel populations are found immediately below the dams where the currents are swift. Although sediment has not significantly reduced flood control storage capacity, there is too much silt for most mussel species of commercial importance.

AGE AND PROPAGATION STUDIES

The age of mussels is difficult to determine because of the lack of true annuli. But mussels do have growth lines, and "the various writers agree that the rings in the shells of freshwater mussels are caused by cessation in growth and that rings formed during the winter period are, in the main, heavier and better marked" (Chamber-

lain, 1931). Growth-like lines may also be caused by excessive handling or any injury that causes the mantle to retract from the edge of the shell. Resumption of growth and duplication of layers of periostracum produce dark "growth rings," according to Coker et al. (1921).

To assess the relative age structure found in the populations of the 2 most important commercial species, *Fusconaea ebenus* and *Pleurobema cordatum*, samples were collected below the Kentucky Dam, in Kentucky Reservoir, and below Pickwick Dam. Measurement of 206 *Fusconaia ebenus* (Table 4) showed that 94% did not have more than 10 growth lines. This species has been reproducing successfully during the past 10 years, especially in the Pickwick Dam tailwater[3] to well below Savannah (TRM 189 area), Tennessee, and in Kentucky Dam tailwater. Measurement of 48 *Pleurobema cordatum* from the same are (Table 5) showed 67% with 10 growth lines or less. Although the latter sample is small, it indicates that this species too has reproduced successfully during the past decade.

Conversely, Scruggs (1960) noted in his study of a large *Pleurobema cordatum* population in Wheeler Reservoir that 90% were older than 12 years and that 20 years had elapsed since the dominant year class originated. He was dealing with an area environmentally unsuitable for the replacement of mussel stocks. That the population was terminal, was indeed proved by the absence of mussels on the Triana bed during the present study. This point is made to emphasize that the tailwater habitats are essentially the only areas that now produce *Fusconaia ebenus* and *Pleurobema cordatum*, and the only areas where they can reproduce successfully.

To gain some information on the unknown details in the life cycles of these naiads, in particular on the fish hosts of their parasitic larval forms, over 300 fish of 28 species were collected over mussel beds near Diamond Island (TRM 195-196) in Pickwick Dam tailwater during the 1964 mussel spawning season. Fish gills were examined and glochidia were found on gills of 7 species: black crappie, spotted sucker, channel catfish, blue catfish, redhorse sucker, flathead catfish and redear sunfish. Because encystments were few and difficult to remove intact, glochidia were not identified as to species.

Experiments on the artificial propagation of *Pleurobema cordatum* and *Fusconaia ebenus* were started in 1965, with white crappie and black crappie as potential host fish. Gravid mussels were placed with the fish in a continuous-flow system but no gill or other encystments were obtained. Failure of this experiment may have been due to the low temperature of the water which did not exceed 70°F except for a few days during the test period. Observation of glochidia in the gravid mussels indicated that development of glochidia had stopped or was retarded when they were placed in the tanks. These studies are being continued under the sponsorship of TVA at a nearby location in the Tennessee River[4].

INVENTORY BY RESERVOIRS

Over 500 miles of the Tennessee River, i.e., all of the lower and part of the upper Tennessee River, were examined in this survey. Because the reservoirs vary in age and physiography, each should be considered an independent ecological unit. Variation is especially evident in the bottom habitats, less so in the chemistry and water

[3]Tailwater, as used in this report, means the portion of the river below a dam that is confined to the original channel.

[4]By Dr. Paul Yokley Jr., Florence State University, Florence, Alabama.

TABLE 8. Mussels found at various sites in the Tennessee River from Pickwick Reservoir to the Nickajack Dam Site (TRM 226-424.7)

Species	Pickwick Reservoir (1963)	Sevenmile Island Area Muscle Shoals Wilson Dam Tailwater of Pickwick Reservoir (1964)*	Wheeler Reservoir and Guntersville Dam Tailwater (1963-64)	Nickajack Dam Site (1965)
	TRM 226-255	TRM 247-253 approx.	TRM 284-348.5	TRM 424.7
Margaritiferidae				
Cumberlandia monodonta	-	X	-	-
Unionidae				
Unioninae				
Fusconaia ebenus	(X)	X	X	-
Fusconaia subrotunda	-	X	-	-
Megalonaias gigantea	(X)	X	X	X
Amblema costata	X	X	X	(X)
Quadrula quadrula	X	X	X	-
Quadrula pustulosa	(X)	X	X	X
Quadrula metanevra	(X)	X	X	X
Tritogonia verrucosa	-	X	X	(X)
Cyclonaias tuberculata granifera	(X)	X	X	(X)
Plethobasus cyphyus	-	X	-	-
Plethobasus cicatricosus	-	X	-	-
Plethobasus cooperianus	-	X	-	-
*Lexingtonia dolabelloides***	-	X	-	-
Pleurobema cordatum	(X)	X	X	X
*Pleurobema oviforme***	-	X	-	-
Elliptio crassidens	(X)	X	X	(X)
Elliptio dilatatus	-	X	X	X

Table 8. (contd.)

Species	Pickwick Reservoir (1963)	Sevenmile Island Area Muscle Shoals Wilson Dam Tailwater of Pickwick Reservoir (1964)*	Wheeler Reservoir and Guntersville Dam Tailwater (1963-64)	Nickajack Dam Site (1965)
	TRM 226-255	TRM 247-253 approx.	TRM 284-348.5	TRM 424.7
Anodontinae				
Anodonta grandis	-	X	-	-
Anodonta suborbiculata	-	X	-	-
Lampsilinae				
Ptychobranchus fasciolaris	-	X	-	-
Obliquaria reflexa	(X)	X	X	(X)
Cyprogenia irrorata	-	X	X	(X)
Obovaria olivaria	-	X	-	-
Actinonaias carinata	-	-	X	-
Plagiola lineolata	-	X	X	X
Leptodea fragilis	-	-	-	X
Proptera alata	(X)	X	X	X
Proptera laevissima	-	X	-	-
Ligumia recta latissima	-	X	-	X
Lampsilis anodontoides	-	X	-	-
Lampsilis ovata	-	X	X	X
Lampsilis orbiculata	(X)	X	X	X

*Collected by Stansbery, 1964.
**Collected by Yokley and Isom, 1965.
***Cumberlandian species.
( ) = valves only
TRM = Tennessee River Miles

quality.

Methods

Exploratory samples were taken across the river channel at 1 mile intervals in the reservoirs and closer together below the dams.[5] This sampling, together with observation of mussel harvesting boats, determined the location and extent of mussel beds.

All bottom sampling was done with a modified Petersen-type dredge. The dredge was constructed of cold-rolled steel and was lead weighted. Steel tines on the biting edge aided in the collection of mussels. Dredge dimensions were 2×1.5 ft; thus each sample covered approximately $\frac{1}{3}$ square yard. The 3,000 samples taken during the survey with the modified dredge corresponded to approximately 10,000 typical Petersen samples. A power winch was used to raise the dredge.

Once mussel beds were located they were divided into $\frac{1}{10}$ square mile quadrats. Randomly selected areas were then sampled intensively, for example 300 quantitative samples were taken in a 12.4 mile reach below Kentucky Dam, and 456 such samples in a 3.5 mile reach below Guntersville Dam, etc.

In the rapidly moving waters below the dams it was found practical to take the following steps for lowering the dredge from the motorboat:

1) direct the bow of the boat into the current;
2) ease forward slowly to gain enough momentum to counteract the current;
3) place the motor in neutral;
4) lower the dredge rapidly, and
5) so direct the dredge that it will arrive at the bottom with the boat directly overhead.

There was little difficulty in getting the sampler to close, as often happens with a typical Petersen sampler. The thin steel walls (about $\frac{1}{4}$ inch) of the modified dredge were sharp and less likely to be wedged open by stones.

Many mussel specimens were donated by mussel fishermen along the river; this aided greatly in getting more species and distribution records.

A few qualitative samples were taken with brails, i.e. commercial mussel fishing gear consisting of barbless wire hooks, below Kentucky Dam.

Records of all specimens collected, both live and dead (shell only), were made in the field. Live material was preserved or taken directly to the laboratory for study.

Results

The present survey revealed the presence of 44 species, including 2 Cumberlandian forms (see Table 2). Results are presented from downstream up.

Kentucky Dam Tailwater (TRM 0-22.4)

Mussel habitat below Kentucky Dam has not been adversely affected by the dam or by maintenance of a 9-ft. deep navigation channel (maintained by flow) from the dam to the mouth of the river.

Most of the naiads in this tailwater are located between TRM 9.6 and 22. Mussels were taken as far downstream as TRM 2, but much of the substrate between TRM 2 and 9.6 is shifting sand and not readily inhabited by naiads. In this tailwater 300 quantitative samples yielded 13 species; 7 other species were taken by brail or collected from mussel fishermen in the area (Table 6).

There were 2.5 mussels per square yard below Kentucky Dam. The principal commercial species are *Fusconaia ebenus* and *Pleurobema cordatum*, the first being the most abundant. Commerical harvesting has been limited here since 1963 because of severe erosion of the shell umbos. The cause of the erosion is unknown.

[5]Only the preimpoundment river channel was sampled extensively and quantitatively.

TABLE 9. Mussels found in Guntersville Dam Tailwater (TRM 345-348.5)

Species	Estimated number per square yard			
	1963		1964	
	Live	Dead Shells	Live	Dead Shells
Megalonaias gigantea	0.203	0.095	0.013	0.013
Amblema costata	-	0.054	0.218	0.090
Quadrula quadrula	0.081	-	-	0.013
Quadrula pustulosa	0.324	0.216	0.346	0.385
Tritogonia verrucosa	0.081	0.014	0.038	0.064
Cyclonaias tuberculata granifera	0.230	0.203	0.282	0.026
Pleurobema cordatum	0.500	0.190	0.462	0.256
Elliptio crassidens	0.122	0.176	0.141	0.128
Elliptio dilatatus	-	0.068	-	-
Obliquaria reflexa	0.689	0.351	0.551	0.397
Cyprogenia irrorata	0.054	-	0.013	-
Actinonaias carinata	0.014	0.027	-	-
Plagiola lineolata	0.068	0.095	0.115	0.077
Proptera alata	-	-	-	0.026
Lampsilis orbiculata	*	-	-	-
Lampsilis ovata	-	-	-	0.013

*Not quantitatively determined but rare.

TABLE 10. Mussels found in Chickamauga Dam tailwater, Chickamauga Reservoir and Watts Bar Dam tailwater (1964)

Species	Chickamauga Dam Tailwater TRM 468-471	Chickamauga Reservoir and Watts Bar Tailwater
Quadrula pustulosa	0.064	0.034
Quadrula metanevra	0.041	-
Cyclonaias tuberculata granifera	0.043	0.023
Pleurobema cordatum	*	0.057
Elliptio crassidens	0.085	0.034
Elliptio dilatatus	-	0.011
Obliquaria reflexa	0.106	0.011
Plagiola lineolata	0.043	-
Proptera alata	0.027	0.011

**Pleurobema cordatum* was the principal commercial shell here in the past; however, no specimens were taken in samples.

Kentucky Reservoir and Pickwick Dam Tailwater (TRM 22.4-206.7)

Kentucky Dam is the newest of the major dams on the Tennessee River. This is reflected in the reservoir's mussel fauna. Kentucky Reservoir species are relatively diversified and widely distributed, but are gradually being restricted to the upper section of the impoundment, primarily because of sediment. The channel at TRM 31.7, 9.3 miles above Kentucky Dam, has an average 4 feet of sediment. From TRM 25 to 111 it consists of fine erosion silt. Above TRM 120 sediment becomes negligible and is primarily sand and gravel. However, there is noticeable fine sediment near the channel shoreline in some upstream areas.

Sediment depth closely parallels the depletion of the mussel fauna in Kentucky Reservoir. For example, only a single empty shell of *Fusconaia ebenus* was found in the samples taken in the TRM 41 area. Reportedly a productive mussel bed before impoundment, this area now has an average of 2 feet of sediment. One live *Megalonaias gigantea* and 1 empty shell of *Pleurobema cordatum* were taken at TRM 60. The substrate in this sample was mud over gravel.

One live *Pleurobema cordatum*, 2 *Megalonaias gigantea* and 1 *Quadrula quadrula* were taken at TRM 71.5 from a mud-laden sample. The small relict population at this station will dissipate as it reaches terminal age, is harvested, or is covered with sediment. There is no evidence of recent recruitment. A small bed at TRM 85 was worked in 1963, but not in April 1964.

The first evidence of a "flowing water" fauna: bryozoa, sponges, snails and caddisflies, was found above TRM 89. The area between TRM 89 and 96 still has a substantial naiad fauna and some mussels were harvested here in 1964. However, since sediment depth varies from 0.2-1 foot, this habitat does not lend itself to rehabilitation. It is, moreover, affected by local industrial pollution below New Johnsonville, Tennessee; this pollution, though, is expected to be brought under control soon by abatement procedures now in progress.

During the 1964 survey there was some harvesting in the Rockport Landing area between TRM 104 and Duck River which joins the Tennessee at TRM 110.8. Sampling showed a very meager and scattered mussel population.

Above Duck River there are a number of commercially important mussel beds, primarily in the area of Roberts Creek (TRM 112.4), Cuba Landing (TRM 114.7), Toms Creek (TRM 124.1), Brodies Landing (TRM 128.2), Perryville Bridge (TRM 134.9), Jennings Bluff (TRM 137.9), Beech Creek Island (TRM 154), Clifton (TRM 157.8), Saltillo (TRM 172), Cerro Gordo (TRM 134.9), and from Chalk Bluff to Pickwick Dam (TRM 185-205). The most apparent cause of mussel decline on these beds is overharvesting.

The sampling was not designed to measure harvest rate, but overharvest is strongly indicated by the marked decrease in yield since 1960 and by the complaints of mussel harvesters. Samples showed that the above beds contain a stock of older mussels. Most evidence of recent reproduction is in the area between TRM 185 and 202, i.e. just below Pickwick Dam.

Quantitative sampling in Kentucky Reservoir revealed 20 naiad species (Table 6). The presence of 5 further species was ascertained as follows: *Obovaria retusa* was found on boats near Saltillo, and one valve of *Pleurobema oviforme*[6] came from a boat at Walkers Landing (TRM 195.5). Exploratory sampling yielded *Cumberlandia monodonta* and *Arcidens confragosus* near TRM 136 and *Obovaria olivaria* near Savannah.

[6]The presence of this specimen cannot be explained in terms of known distribution records.

In addition, Bates (1962) reported *Leptodea laevissima*, *L. fragilis*, *Carunculina parva*, *Anodonta corpulenta* and *A. suborbiculata* from Kentucky Reservoir, which adds up to a total of 30 species.

Based on the survey data, the Kentucky Reservoir mussel population is estimated at about 62 million animals; *Fusconaia ebenus* accounts for 58% of the population or about 36 million animals (see Table 7 for a comparison of the abundance of dominant species in the various portions of the lower Tennessee River); *Pleurobema cordatum* ranks second in abundance, with 10 million; *Obliquaria reflexa* third with 8 million, and *Quadrula pustulosa* fourth with 6 million animals. Together these 4 species make up almost 97% of the entire mussel population of Kentucky Reservoir and Pickwick dam tailwater.

Fusconaia ebenus gained dominance in Kentucky Reservoir only recently. Scruggs (1960) reported *Pleurobema cordatum* as most abundant in the 1957 harvest and TVA records show *Pleurobema* dominant in each subsequent year's harvest until 1963, when *Fusconaia ebenus* outnumbered it 2:1 for the first time.

As further evidence that *Fusconaia ebenus* is better able to cope with the changing environment, 12% only of the recovered shells of this species consisted of valves only as compared with 40% for *Pleurobema cordatum*. Comparison of Tables 4 and 5 also shows that *Fusconaia ebenus* is reproducing more successfully in recent years than *Pleurobema cordatum*.

The 1964 shell harvest in Kentucky Reservoir was 64% less than in 1963. But even with this drastic decline, Kentucky Reservoir still produced 1,253 tons of shells, more than any other area in the Valley. The 95-mile stretch of river channel between Duck River and Pickwick Dam is the most promising future source of shells for the Valley shell industry. *Fusconaia ebenus* promises to be the most important species, but *Quadrula quadrula*, reported by Bates (1962) to have successfully adapted to the shallow regions of Kentucky Reservoir may also contribute to future mussel harvests.

Pickwick Reservoir and Wilson Dam Tailwater (TRM 206.7-259.4)

The 79 samples taken in Pickwick Reservoir in the fall of 1963 yielded shells of 12 species but only 2 live specimens among them: 1 *Quadrula quadrula* and 1 *Amblema costata* (Table 8). Most of the samples had been taken in the reservoir proper, where sediment has been a major factor in the depletion of mussels.

Stansbery (1964) reported 30 species from the Wilson Dam tailwater portion of Pickwick Reservoir (lower "Muscle Shoals", Sevenmile Island area, TRM 247-253) for 1963. To this fauna must be added another species, *Cumberlandia monodonta*, collected in 1965 (Table 8).

A few shells were harvested commercially in that same area in 1963, but their quality was poor and the operation was discontinued. Harvesting was resumed in 1965 on a very limited basis and for short duration.

Commercial species are now restricted to about 12 miles from Wilson Dam to just below Sevenmile Island. This portion of the river should continue to produce mussels. *Pleurobema cordatum* is the principal commercial species there. This area lies within the terminal 25-mile portion of a 53-mile stretch that was known as the "Muscle Shoals" (TRM 234.6-287.7), sometimes incorrectly referred to as "Mussel Shoals", formerly one of the finest mussel habitats known to man. The remainder now lies at the bottoms of Wilson and Wheeler Reservoirs. Because of the unusual interest of the region, it will be treated in greater detail.

Muscle Shoals

Although the Tennessee River in north Alabama flows over St. Louis limestone,

a faint minor swell "brings up the hard Fort Payne chert above the bed of the river for 35 miles above Florence, Alabama, causing rapids and falls throughout this distance, with a total fall of 140 feet" (Fenneman, 1938). Harris and co-authors (1963) report an alternating sequence of chert and limestone strata for about 30 miles downstream of Wilson Dam, i.e. in that same stretch of the river.

Mean gradients in feet per mile for some representative areas between Pickwick and Wheeler Dams are (in parentheses): TRM 209.2-229.80 (1.19), TRM 245.9-258.2 (2.23), TRM 260.0-272.9 (4.3).

According to Droze (1965): "The region of the Tennessee River known as the Muscle shoals was in reality a series of shoals with intervening pools of deep water. . . moving downstream, one encountered first the Elk River shoals, second the Big Muscle Shoals, third the Little Muscle Shoals, and finally the Colbert Shoals . . ."

Referring to the shoals and their naiad fauna, van der Schalie (1939) said: "The wide range of ecological conditions afforded by the intrusion of shoals in this portion of the lower Tennessee has resulted in the maintenance at Muscle Shoals of the largest number of species found anywhere." He listed 40 species from "recent records." Ortman (1925) had listed 69 naiad species for the shoals. Stansbery (1964) found only 30 species in collections made on the lower shoals in 1963. In 1965 Yokley and the author added 1 further species (Table 8), bringing the total up to 31 species. Recently 44 species were recorded in this area (Yokley,[7] pers. comm., 1968), including 4 additional species of Cumberlandian origin; many of these species are rare.

Pickwick Dam Reservoir's effect on the mussel fauna is evident. Prior to impoundment, Muscle Shoals was inhabited by "many species that commonly inhabit creeks and shallow rivers" (van der Schalie, 1939). In Ortmann's (1924b) view "the cause for this unusual development of Naiad-life (as well as other freshwater life) of this region is found in the fact that here two old faunas, in themselves exceptionally rich, come together," the Cumberlandian and that of the 'Interior Basin'. He also noted that T. A. Conrad had called attention to this abundance of naiad species at Muscle Shoals as early as 1834.

Whereas Stansbery's investigation (1964) and the present study revealed no more than 2 Cumberlandian species remaining on the shoals, *Lexingtonia dolabelloides* and *Pleurobema oviforme*, Yokley, as mentioned above, has obtained 4 additional records for this group.

Prior to impoundment of Pickwick Reservoir the Cumberlandian species did not extend downstream beyond the Muscle Shoals. Van der Schalie (1939) had suggested that the presence of the Fall Line[8] and of the Tuscaloosa Group, a geological formation composed of stratified gravel containing lenses of clay and silty sand in the vicinity of Bear Creek (Riverton, Alabama, TRM 226) area, might be the barrier to the downstream extension of Cumberlandian mussels and to the upstream extension of some Interior Basin species. However, Isom & Yokley (1968), in a study of Bear Creek, found Cumberlandian species. Bear Creek is almost totally within influence of the Tuscaloosa Group, which shows that the stratum in itself is not limiting to these forms. Ortmann (1925) also recorded Cumberlandian

[7]By Dr. Paul Yokley Jr., Florence State University, Florence, Alabama.

[8]The Fall Line here is the juncture of the East Gulf Coastal Plain section of the Coastal Plain physiographic province and the Interior Low Plateau province, and refers to the position at which it crosses the Tennessee River (Fenneman, 1938).

species for the drainage.

Van der Schalie's (1939) faunal list indicated the absence of 2 Interior Basin species above Muscle Shoals, *Fusconaia ebenus* and *Quadrula quadrula*. However, Scruggs (1960) has shown, and the present study has confirmed, that these species do now occur above the shoals. *Fusconaia ebenus* and *Quadrula quadrula* have been found as far upstream as Chickamauga Reservoir. It is possible that the conditions in the upper river have changed sufficiently since impoundment to be within the tolerance limits of these mussels, of their fish hosts, or both.

In summary then, of the 25 miles of the Muscle Shoals below Wilson Dam, approximately 12 miles (TRM 247-259.4) present an environment suitable for mussels. This is true even though a 9-foot navigation channel is maintained by water level management and flow regulation.

This study has revealed that at least 31 species of mussels still inhabit the lower Muscle Shoals (Table 8). Of these, 2 are of Cumberlandian origin while the remaining 29 are of Interior Basin or unknown origin. The major change has been the decline in the number of Cumberlandian species which formerly numbered 25 (Ortmann, 1925).

Wilson Reservoir (TRM 259.4-274.9)

A few samples taken in a transect across Wilson Reservoir at the site of the Shoal Creek shoals in the fall of 1964 yielded no naiads. This portion of the Muscle Shoals was one of the finest mussel habitats known prior to impoundment in 1924.

Wheeler Reservoir and Guntersville Dam Tailwater (TRM 274.9-349.0)

Wheeler Reservoir sampling in 1963 demonstrated a population of *Pleurobema cordatum*, *Megalonaias gigantea* and *Amblema costata* in the area between TRM 289 and 300. But this population seems to be terminal; there is no evidence of recent reproduction. The bottom here is covered with sediment.

Three dredge boats of the type used in oyster harvesting took 200 tons of shells in this area during the summer and fall of 1963. The *Pleurobema cordatum* shells were thin and of very poor quality, while the quality of *Megalonaias gigantea* and *Amblema costata* shells was relatively good. A check of the dredge boats in October 1963 revealed a few live mussels being taken along with many empty valves. This population will dissipate as the old year classes are harvested or reach terminal age.

Scruggs (1960) in his study of *Pleurobema cordatum* estimated a mean population of 20,566,000 on the Triana bed of this reservoir (TRM 308-316). Mussel beds between Decatur, Alabama, and Indian Creek (TRM 304.1-320.8), below Triana Landing, were harvested heavily in 1956 and 1957. Scruggs reported: The harvesting rate for *Pleurobema* on beds in Wheeler Reservoir during a 2-year period (1956-57) accounted for almost 23% of the available population. Recruitment to the population during this period amounted to less than 1%. Only 1 live specimen was taken in this area during the present survey.

Harvesting had ceased on the Triana bed 4 years after Scruggs' study. He attributed the decline of the population to the "high rate of exploitation" and the unfavorable environmental conditions created by impoundment. In addition to overharvesting and the effects of sediment on this bed, industrial waste entering the Tennessee River by way of Indian Creek (TRM 320.8) may have also contributed to the population decline.

The present study indicates only a small mussel population immediately above Triana. The first productive mussel bed was found at TRM 331 in the Guntersville Dam tailwater. This bed is almost continuous up to TRM 348, but the entire area is overharvested and only between TRM 345 and Guntersville

Dam (TRM 349) is it unaffected by sediment.

Guntersville Dam tailwater and the Sevenmile Island area below Wilson Dam have the last concentrations of commercially important mussels in north Alabama. Most of the shells harvested in north Alabama in 1963 and 1964 came from Guntersville Dam tailwater, between TRM 345 and 348.5.

Quantitative sampling in this tailwater in 1963 and again in 1964 included three $\frac{1}{3}$-square-yard samples per $\frac{1}{10}$-square mile of bed area. Rock substrate above TRM 348.5 does not support mussels in the navigation channel or along the left bank. However, the mussel bed continues up the right bank to TRM 348. The 222 samples taken in 1963 (Table 9) yielded 191 live mussels and 105 shells. *Obliquaria reflexa* was most abundant with an average density of 0.69 per square yard; next came *Pleurobema cordatum* and *Quadrula pustulosa*, with estimated densities of 0.50 and 0.32 respectively. The 234 samples in 1964 showed a similar distribution, the estimated densities per square yard of these 3 most abundant species being 0.55, 0.46 and 0.35. Sampling error (±1 standard deviation) for all samples in which live mussels were taken was 9.1% in 1963 and 9.0% in 1964.

Overharvesting is the most apparent cause of population decline in Guntersville Dam tailwater. Moreover, there is little evidence of substantial recent recruitment. *Pleurobema cordatum* is the species of principal commercial importance here.

Guntersville Reservoir and Hales Bar Tailwater (TRM 349-431.1)

No live mussels were found in the 150 samples taken in Guntersville Reservoir and Hales Bar tailwater in 1964. In the preimpoundment river channel portion of this reservoir, sedimentation is sufficient to prevent re-establishment of commercial species.

Commercial fishermen took a few mussels from Hales Bar tailwater early in 1963 with brails and a few more in the reservoir with dredges, but since then there has been no brail harvest in Guntersville Reservoir. *Pleurobema cordatum* was the principal commercial mussel in the past.

There is, however, a substantial invasion of shallows parallel to the river channel by *Quadrula quadrula* and *Amblema costata*. These species were discovered when Guntersville Reservoir was lowered during an aquatic plant (*Myriophyllum spicatum*) control program in the fall of 1964. During the few weeks the water level was down, mussel harvesters picked up 70 tons of shells of these 2 species, or roughly 140,000 mussels.

An unusual opportunity for collecting mollusks occurred in 1965 when the construction site for the Nickajack Dam was drained. This site is at TRM 424.7 or 6.4 miles below Hales Bar Dam which Nickajack Dam has now replaced. A diversion canal around the dam and lock site made a 1,700-foot-long section of the river accessible to collecting. On June 16, 1965, 11 species were collected alive along with shells of 6 other species (Table 8). Mussel species were scattered over the whole river bed except for *Megalonaias gigantea* which was abundant only along the left bank near the upstream cofferdam.

Hales Bar Reservoir and Chickamauga Dam Tailwater (TRM 431.1-471)

Hales Bar Reservoir was sampled in March 1964. Sampling began just above the dam (TRM 432) and terminated below Chickamauga Dam (TRM 470). Mussels found in the 141 samples and their densities are shown in Table 10.

Mussel beds below Chickamauga Dam and South Chickamauga Creek (TRM 468.2) that produced good harvests in 1963 are now virtually gone. In addition to exploitation, pollution may be affecting these beds. It comes from barges, sewage and industrial wastes and is most severe below South Chickamuga

Creek. A survey by the Tennessee Stream Pollution Control Board, 1964, notes other sources of pollution in the Chattanooga area. In that stretch high mortality was noted among the introduced Asiatic clams (*Corbicula manillensis*); dead mussels and a few dead fish were also taken in the samples.

The future of Hales Bar Reservoir mussel production depends on some 100 acres of suitable substrate between South Chickamauga Creek and Chickamauga Dam, TRM 468.25-470. If pollution between Moccasin Bend (TRM 458) and South Chickamauga Creek is abated, mussels may repopulate this area, but it would be many years before a population of commercial-size specimens could be hoped for under the best of circumstances.

Divers took a few shells below and above South Chickamauga Creek in 1964, but there has been no brail harvesting since 1963. Here also *Pleurobema cordatum* was the principal commercial species taken in past years. A state proclamation closed Hales Bar Reservoir to mussel harvesters on July 1, 1965.

Chickamauga Reservoir and Watts Bar Tailwater (TRM 471.0-529.9)

Chickamauga Reservoir, from TRM 500 to Watts Bar Dam, contains a substantial population of commercial mussels, principally *Pleurobema cordatum* (Table 10). However, the population is now much smaller than that found by Scruggs (1960) in 1956-57. He noted a total population averaging 16.7 individuals per square yard. The present survey, in 1964, showed less than 1 specimen per square yard.

The substrate in Watts Bar tailwater was difficult to sample. The river bed here is principally bedrock, the interstices of which are filled with gravel, rock, clay and other sediment. Sampling error was 32.5% based on all samples in which live mussels were taken.

Sediment in lower and middle Chickamauga Reservoir and overharvesting in Watts Bar tailwater are apparently responsible for the decline of the population. Water quality is generally good. Regulatory measures instituted by the State of Tennessee will help to sustain the mussel fishery of Watts Bar tailwater.

Chickamauga Reservoir was lowered in January 1966 to control Eurasian milfoil (*Myriophyllum spicatum*). Examination of exposed areas revealed extensive populations of *Anodonta grandis*, *A. suborbiculata* and specimens of *Lasmigona complanata*, all new distribution records. However, *Quadrula quadrula* and *Amblema costata*, both found in like situations in Guntersville Reservoir, were not found in Chickamauga.

MANAGEMENT ACTION AND FUTURE NEEDS

Representatives of the TVA, Alabama Department of Conservation, Kentucky Department of Fish and Wildlife Resources, Tennessee Game and Fish Commission, major shell companies and other interested citizens met on August 18, 1964, to discuss the results of the mussel resource inventory just concluded. The TVA suggested harvest regulations and everyone, including the shell companies, agreed that this was a necessary first step toward rebuilding the depleting resource.

Tennessee passed enabling legislation in 1965 and issued a proclamation, effective from July 1, governing commercial mussel harvest. Sanctuaries have been designated where musseling and other activities detrimental to mussels are prohibited. Mussel harvesting season is open the year round, but mussels can be taken only between sunrise and sunset. Specimens less than 2½ inches in diameter must be

returned to the water. Harvesting may be by brail or by hand (in shallow streams) but not by mechanized (or hydraulic) dredges. Effective from July 1, 1966, brail bars shall be no longer than 16 feet; brail hooks must be of 14 gage wire or larger, and prongs can be no longer than 1¼ inches. Mussel fishermen and buyers must be licensed.

Kentucky issued a similar proclamation effective from January 1, 1966. In the fall of 1966 the Alabama Legislature adopted a bill giving the Department of Conservation authority to regulate mussel harvest. An interstate compact or similar cooperative arrangement between the mussel producing states in the Mississippi Valley would help ensure consistent conservation measures and perhaps prevent overexploitation of any one stream.

Regulation of the mussel harvest will not solve all of the problems overnight. But regulation and establishment of protected areas will help to halt the rapid population decline. Recent estimates of Japanese cultured-pearl industry requirements run to about 3,000 tons per year. The Tennessee Valley might supply ⅓ of this requirement in the years immediately ahead. Hopefully, it might eventually supply the total 3,000 tons on a sustained basis.

In the meantime, the mussel industry might well explore the possibility of establishing freshwater cultured-pearl farms. The long-range studies needed to unravel the life histories of *Pleurobema cordatum* and *Fusconaia ebenus* should be continued and more study is likewise needed on the species that have now become established in Tennessee River impoundments, especially *Quadrula quadrula*. With the regulation of mussel harvest, state game and fish agencies should now assume responsibility for collecting data regarding annual mussel harvest and for evaluating the effectiveness of control measures.

DISCUSSION AND SUMMARY

Habitats suitable for most naiads are now found in about 175 miles of tailwater below the dams, distributed as follows, starting upstream: 29 miles below Watts Bar, 3 miles below Chickamauga, 10 miles below Hales Bar, 15 miles below Guntersville, 12 miles below Wilson, 94 miles below Pickwick, and 13 miles below Kentucky Dam.

The original extent of mussel beds in the Tennessee River is essentially unknown since it was never before surveyed in its entirety. Intermittent stretches of bottom habitat unsuitable for mussels, i.e., bedrock and mud-gravel, undoubtedly occurred, as they do in any stream. The stretch occupied by commercially operable beds therefore was certainly less than 529 miles, i.e., the length surveyed, and more than 175 miles, the length still suitable, probably at the least 350 miles.

The 1965 mussel population in the 175 miles thought to provide a suitable habitat is roughly estimated at 26,000 tons. The 2 top commercial species, *Pleurobema cordatum* and *Fusconaia ebenus*, account for about 1/3 of the populations. Other commercial species, *Megalonaias gigantea*, *Amblema costata*, *Quadrula quadrula*, *Q. pustulosa* and *Plagiola lineolata*, make up another third, while colored, irregularly shaped and thinshelled noncommercial species account for the balance. This means that only about 17,000 tons of commercial shells are available to maintain the industry for the next few years, with a marginal yield of perhaps 1000 tons annually.

As a result of the present investigation certain factors could be eliminated as contributing causes of the mussel decline. For example, water quality in the Tennessee River is generally good; there was no indication that pollution or otherwise unsatisfactory water

conditions were responsible for the reductions observed, except in limited areas. Neither was any evidence of disease or extensive parasitism found in live mussels. On the other hand, overharvesting is confirmed as one of the reasons for the decline throughout the river and especially in the tailwaters below the dams where a suitable habitat still exists. Age and growth studies indicate that only in the 30-40 miles below Pickwick Dam has there been substantial recent reproduction and survival of the young of commercially important species. In the absence of natural replacement, or wherever replacement rates are lower than harvest rates, certain tailwater mussel beds have been gradually depleted by heavy continuing harvest.

However, overharvesting is neither the sole nor the primary cause of mussel decline, but alteration of environment where beds existing before impoundment are now covered by slack water. Here a once suitable habitat has been changed by slow-moving current and the ensuing deposition of silt. These conditions affect the survival of young mussels, causing their inevitable disappearance. Restrictions in these areas would not be effective in restoring mussels to earlier levels of abundance.

In these sections of the Tennessee River, then, mussel populations have probably declined for many more years than was evident from harvest data. Annual changes in market demand and shell prices graphically reflect a series of peaks and declines in effort expended and in total harvest. The effects of these fluctuations apparently masked a general decline that has been under way since environmental changes became severe enough on some beds to effectively prevent the survival of young mussels.

Principal changes in the mussel fauna distribution and composition of the Tennessee River since impoundment consist in a) the colonization of shallows by some species, b) the intrusion of species unreported prior to impoundment, and reduction in number or elimination of other species.

As regards the settlement in the shallows: *Megalonaias gigantea*, *Arcidens confragosus*, *Anodonta corpulenta*, *A. grandis*, *A. imbecillis* and *Carunculina parva* are now common in Kentucky Reservoir; *Quadrula quadrula* and *Anodonta grandis* have been taken from Pickwick Reservoir; *Quadrula quadrula* has become established in Wheeler Reservoir; *Amblema costata* and *Quadrula quadrula* are common in Guntersville Reservoir. *Anodonta suborbiculata*, *A. grandis* and *Lasmigona complanata* were taken from Chickamauga Reservoir.

To sum up the various faunal shifts from a different viewpoint: while some Unioninae, especially *Quadrula quadrula* and *Amblema costata* have successfully established themselves in the overbank habitats provided by impoundment, others, *Fusconaia ebenus*, *Quadrula pustulosa* and *Pleurobema cordatum* have become increasingly rare, even though *F. ebenus* seems lately to have gained dominance in Kentucky Reservoir. Anodontinae, formerly reported as entirely absent from the lower Tennessee River (van der Schalie, 1939) have become widespread in most reservoirs and include *Arcidens congragosus*, *Lasmigona complanata*, *Anodonta grandis*, *A. imbecillis* and *A. suborbiculata*. Bates (1962) reported the following Lampsilinae from mud-shallows in Kentucky Reservoir: *Leptodea laevissima*, *L. fragilis*, *Carunculina parva* and *Truncilla donaciformis;* others have been grossly affected by impoundment; these include: *Dromus dromus*, *Obovaria retusa; Ligumia recta latissima*, to name a few. The range of 3 species seems to have extended upstream since impoundment: *Fusconaia ebenus* and *Quadrula quadrula*, whose absence appears from data given by van der Schalie (1939) and *Megalonaias gigantea*, which van der Schalie (1938) reported as entirely absent from the upper Tennes-

see and yet was the most abundant species at the Nickajack Dam site in 1965.

The Anodontinae *Arcidens congragosus* and *Lasmigona complanata* are new records for the Tennessee River. The latter has been previously reported from one locality in Duck River, a tributary of the lower Tennessee between Pickwick and Kentucky Dams (Ortmann, 1924a), while the former is a new record for the basin.

Many of the 44 naiad species recovered during this survey, and particularly the additional species later collected by Yokley, are rarely found and are apparently disappearing.

The future of the Tennessee River mussel fishery is uncertain. The present 175 miles of suitable habitat should continue to produce mussels, in particular the area below Pickwick Dam; how many and for how long will depend on how the resource is managed. Pollution, even though not a serious problem now, should be controlled. The range of silt-tolerant commercial species and those that have colonized reservoir shallows can probably be extended. The many unknown aspects of the life history of *Pleurobema cordatum* and *Fusconaia ebenus*, the species of greatest commercial importance, will have to be elucidated before these species can be successfully managed.

As a result of the TVA investigation, Tennessee and Kentucky game and fish agencies adopted harvest regulations in 1965 to conserve and rebuild mussel populations. The Alabama Legislature passed enabling legislation in 1966. Adoption of uniform harvesting regulations by the 3 states involved and establishment of sanctuary sections in each tailwater will go far toward protecting and strengthening the remaining naiad populations.

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RÉSUMÉ

LES RESSOURCES MOULIÈRES DE LA RIVIÈRE TENNESSEE

B. G. Isom

La présente étude tente d'établir le statut de la faune moulière de la rivière Tennessee pour 1965 et de la comparer à la riche faune endémique qui existait antérieurement à l'aménagement de la rivière par la "Tennessee Valley Authority" en 1936. Beaucoup d'espèces ont eu de l'importance dans le passé pour l'industrie du bouton de nacre et quelques unes comptent encore actuellement pour l'industrie des perles de culture.

Les récoltes annuelles d'environ 10.000 tonnes de coquilles de 1940 à 1950 ont régulièrement décliné, tombant jusqu'à 2.000 tonnes de 1964 à 1967. Les populations de moules ont été significativement affectées par l'aménagement, aussi bien en ce qui concerne la composition que la distribution des espèces. Tandis que l'aménagement a été la première cause de déclin par suite de la réduction des habitats propices aux moules, la surexploitation des moules dans les tronçons d'eau-vive au-dessus des barrages a eu récemment pour conséquences un plus rapide épuisement des ressources moulières.

Approximativement 175 miles d'habitat propice demeurent sur les 531 miles d'origine, particulièrement en amont des barrages. La faune actuelle comprend 44 espèces contre environ 100 dénombrées autrefois. La brutale réduction des espèces "Cumberlandiennes" (6 contre 25) est confirmée. Parmi les espèces encore présentes, il y en a beaucoup qui sont devenues rares. Ce n'est que récemment que plusieurs espèces se sont établies dans leurs actuelles localisations.

La législation réglementant la récolte des moules, promulguée en 1965-1966, devrait permettre d'enrayer l'épuisement des ressources moulières. Le domaine des espèces tolérant la vase s'étend graduellement, mais avant que les moules d'intérêt commercial, en particulier *Fusconaia ebenus* et *Pleurobema cordatum*, ne puissent être exploitées avec succès, il sera nécessaire d'élucider leur mode de vie. L'avenir de l'industrie moulière demeure présentement incertain.

RESUMEN

RESERVA DE ALMEJAS EN EL RIO TENNESSEE

B. G. Isom

Este estudio es un esfuerzo para valorizar el estado de las almejas del Río Tennessee, registradas en el año 1965 en comparación a la rica fauna endémica anterior al endicamiento por la Autoridad del Valle del Tennessee en 1936. Muchas especies fueron importantes en el pasado para la industria de botones de nacar, y algunas todavía lo son en la industria de las perlas cultivadas.

La producción, de cerca 10.000 toneladas de conchas en las décadas 1940 y 1950, ha declinado sin interrupción a 2000 toneladas desde 1964 hasta fines de 1967. Las poblaciones de almejas han sido significativamente afectadas por el endicamiento, tanto en la distribución como en los componentes específicos. Aparte de que el endicamiento ha sido la causa principal de esta decadencia, al reducir el habitat de las especies, un más rápido agotamiento de las reservas se debió también a la gran explotación en fechas recientes, de las poblaciones fluviales del dique abajo.

Alrededor de 175 millas de habitat favorable han quedado de las primeras 531 millas del río. La fauna, al presente, comprende 44 especies, en lugar de las 100 de antes. La drástica reducción de especies "Cumberlandianas" (quedan sólo 8 de las 25 que antes existian) se confirma. Muchas de las especies que aun viven se van haciendo más raras, y sólo algunas últimamente llegaron a localizarse en los lugares presentes.

Las leyes promulgadas en 1965-1966 para regular la explotación, podran detener la rápida depleción. El área de la especies tolerantes al limo se va extendiendo gradualmente pero, antes que las especies de importancia comercial, particularmente *Fusconaia ebenus* y *Pleurobema cordatum*, puedan manipularse con éxito, deberán dilucidarse sus ciclos biológicos. Por ahora, el futuro de la industria es incierto.

АБСТРАКТ

ЗАПАСЫ ДВУСТВОРОК В РЕКЕ ТЕННЕССИ

Б. Г. АЙЗОМ

В настоящей статье делается попытка оценить состояние фауны двустворок в реке Теннесси, начиная с 1965 г., и сравнить его с богатой эндемичной фауной моллюсков, существовавшей здесь раньше, до постройки плотины в 1936 г.

Многие виды двустворок раньше имели промысловое значение (как перламутр для пуговичной промышленности), а некоторые из них и сейчас имеют значение для разведения их в промышленном масштабе с целью получения жемчуга.

Годовой вылов, составлявший около 10 тыс. тонн раковин в 1940-1950 гг., стал затем неуклонно падать, составив около 2000 тонн в 1964-67 гг. На видовой состав популяций моллюсков и на их распространение оказало сильное влияние запруживание реки. Если это было первопричиной уменьшения запасов моллюсков из-за сокращения пригодных для них мест обитания, то перелов их в частях реки, ниже плотины в настоящее время приводит к дальнейшему быстрому истощению рессурсов моллюсков. Остается около 175 миль реки (из первых 531 миль, главным образом ниже плотины), пригодных для обитания моллюсков. В настоящее время в фауне их имеется 44 вида, против 100, обитавших здесь раньше. Наблюдается сильное сокращение "кумберландских" видов (осталось 6 из 25). Многие, еще живущие здесь виды стали редкими; некоторые лишь недавно стали встречаться на современных местах обитания.

Закон о регулировании вылова моллюсков, действующий с 1965-66 гг., должен помочь задержать быстрое истощение их запасов. Развитие выносливых к заилению видов постепенно увеличивается, однако прежде чем промысловые формы, особенно *Fusconaia ebenus* и *Pleurobema cordatum* можно будет успешно разводить, необходимо изучить их биологию и жизненный цикл. В настоящее время перспективы промысла моллюсков в реке неясны.

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TECHNIQUES FOR RECOVERING AND IDENTIFYING LARVAE OF *ANGIOSTRONGYLUS CANTONENSIS* FROM MOLLUSCS

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ABSTRACT

Molluscs are obligatory intermediate hosts of *Angiostrongylus cantonensis*, a metastrongylid lungworm of rats, which is responsible for much human disease in the Pacific basin. A variety of techniques for recovering, counting and identifying 3rd-stage larvae were evaluated in the course of examining large numbers of land and freshwater molluscs of various species naturally or experimentally infected with this parasite.

Eventually, 2 basic methods were employed to detect larvae. Smaller molluscs were initially pressed between glass plates and larger species were digested. In connection with the latter technique, experiments were carried out to evaluate methods for filtering, sedimenting and examining the digest. Observations were also made on the proportions of larvae present in the viscera of naturally infected molluscs as compared with the foot, and on recovery rates of adult *Angiostrongylus cantonensis* when rats were fed various numbers of 3rd-stage larvae from different species of naturally infected molluscs.

Angiostrongylus cantonensis (Chen) Dougherty, a metastrongylid lungworm of rats, was described from Canton, China by Chen in 1935. Some 20 years later, Mackerras & Sandars (1955) discovered its unusual migration in vertebrate hosts and established that molluscs were obligatory intermediate hosts. Although *A. cantonensis* was first reported as a parasite of man in 1945 (Beaver & Rosen, 1964), it was not until after 1961, when it was incriminated as a possible etiologic agent of the eosinophilic meningitis seen on Pacific islands (Rosen, et al., 1962), that the true significance of this nematode as a cause of human disease became known.

Many species of gastropods common in the Pacific basin have been found naturally infected with *Angiostrongylus cantonensis* (Punyagupta, 1965; Lim et al., 1965; Wallace & Rosen, 1969). In some localities, more than 50% of certain terrestrial species such as *Achatina fulica* Bowdich, the Giant African Snail, and veronicellid slugs are naturally infected, and individual specimens commonly contain several hundred or more larvae each.

Although considerable data have been published on techniques for examining the molluscan intermediate hosts of the many metastrongylid lungworms, a great variety of techniques have been employed and few technical details have been given - especially in regard to quantitative aspects. In the course of examining large numbers of molluscs of various species naturally or experimentally infected with *Angiostrongylus cantonensis*, we evaluated a variety of techniques for recovering, counting and identifying 3rd-stage larvae. A de-

scription of some of the techniques and their evaluation is given in this report so that the information will be available to malacologists and others who undertake investigations on intermediate hosts of *A. cantonensis* and other metastrongylid nematodes.

MATERIALS, METHODS AND THEIR EVALUATION

Two basic methods were used for detecting and recovering larvae in molluscs. These were, 1) pressing the animal between a pair of glass plates, and 2) artificial digestion.

Pressing

This relatively simple technique was used with small snails in the size range of *Subulina octona* (Bruguiere) (length about 0.5 to 2.0 cm), or with soft-bodied slugs like *Deroceras laeve* (Müller) (Length about 1.0 to 2.5 cm). Glass plates were cut from double-strength high-quality window glass. The bottom plate was about 11.5 cm square, the top plate about 10.0 cm square, and all edges were polished.

The slug or snail, removed from its shell, was placed on the larger of the plates and finely minced with scissors. The smaller plate was then placed over the minced tissue and pressure applied so that the tissue was flattened out evenly. Examination was done with a stereoscopic microscope employing transmitted light and a magnification of 25×. Several snails or slugs could be examined between 1 set of plates. Tissue of molluscs found to harbor nematode larvae was removed from the plates and digested (see below).

Artificial digestion

Digestion techniques. Digestive fluid was prepared in 1000 ml quantities by mixing 7 ml of reagent-grade concentrated hydrochloric acid with 993 ml of warm (40°C) water. Five gms of pepsin powder NF were then added and dissolved with the aid of a magnetic stirrer.

Two methods of digestion were used, depending on the size of the mollusc. Small to medium-sized snails or slugs such as *Subulina octona*, *Bradybaena similaris* (Férussac), *Deroceras laeve* and *Biomphalaria* (=*Australorbis*) *glabrata* (Say) were digested in a petri dish at room temperature with the aid of a magnetic stirring bar. Larger molluscs, such as adult veronicellid slugs and Giant African Snails, *Achatina fulica* Bowdich, were digested in flasks at 37°C with the contents constantly agitated either by a magnetic stirring bar or by a serological rotator.

In the 1st method, the animal was removed from its shell and minced with scissors in a plastic petri dish (either 60 mm × 15 mm or 100 mm × 15 mm). At a room temperature of about 23°C, the temperature in the dish reached 27° or 28°C from the heat of the stirrer and digestion was usually complete within 30 minutes. The digest was left in the same petri dish and examined with a stereoscopic microscope. Tissues of pressed specimens were also digested in petri dishes on a magnetic stirrer. Digestive fluid (in a plastic "squeeze-bottle" with a small bore spout) was used to rinse the tissue off the plates.

The following technique was eventually selected for use with large molluscs. After cracking the shell with a hammer, the entire animal was removed and placed in the jar of a Waring blendor which contained just enough digestive fluid (about 150 ml) to cover the blades. The mouth of the jar was covered by placing aluminum foil under the lid and the blendor ran for about 45 seconds. The foil cover was then discarded and the contents of the jar poured into a 500 ml Erlenmeyer flask. The blendor was rinsed with approximately 100 ml of fresh digestive fluid and this also was added to the flask. Occasionally, a 2nd rinse with digestive fluid was necessary to remove all tissue fragments. To avoid cross contamination of specimens, the blendor jar was thoroughly rinsed with hot water (approximately 60°C)

before re-use.

Additional digestive fluid was not added to the flask unless the mollusc weighed more than 20 gms. In this case, additional fluid was added until the total was about 350 ml. The flask was then placed in a 37°C incubator and the contents agitated as described above. Under these conditions, digestion for 1 hr. was usually sufficient. Larvae were concentrated and recovered from the digest by a modification of a technique described by Baermann (1917). This consisted of filtering the digest in a funnel and then collecting the larvae from the bottom of the funnel several hrs. later.

Funnel and Filters. The funnel was of clear glass and had an angle of 60°, a capacity of 500 ml., a diameter of approximately 15 cm (6 inches) and a stem of the same length. The overall length was 28 cm. A piece of rubber tubing 10 to 23 cm long with a bore of about 1.0 cm was slipped over the end of the stem and closed with a Hoffman screw-compressor clamp. As many as 4 funnels could be arranged on 1 ring stand by adjusting the lengths of the rubber tubing.

Stainless steel gauze, cotton surgical gauze and silk cloth were tested as filters. Stainless steel gauze was self supporting. The cotton gauze or silk was suspended in the top of the funnel by using a piece of brass wire gauze about 15 cm square, shaped to form a support approximately 4 cm below the top of the funnel. The digest fluid was poured into the funnel through the filter, and tap water was added until the level of liquid covered all of the particulate material retained on the filter. Italian silk screen (No. 16) with 157 mesh per linear inch yielded the clearest filtrate but appeared to hold back some larvae. Both stainless steel gauze with a mesh count of 325 × 325 per linear inch (and openings of 0.0017 of an inch) and 4 layers of 28 × 24 medium mesh absorbent cotton surgical gauze appeared to offer little hindrance to larvae and yielded a satisfactory filtrate.

The ability of 3rd-stage larvae of *Angiostrongylus cantonensis* to pass through each of these materials was tested in a series of 3 experiments summarized in Table 1. Each experiment consisted of adding 40 freshly isolated larvae to each of 3 funnels containing water and the respective filter. Aliquots of 22 to 24 ml from the stem of the funnel were collected and examined at hourly intervals for 4 hrs. It will be noted from Table 1 that silk appeared to retain more larvae than either stainless steel or cotton gauze.

Quantity of fluid examined and settling time. Either 60 mm × 15 mm or 100 mm × 15 mm plastic petri dishes were used to collect and examine fluid from funnels. The dishes were filled to the desired capacity by placing them directly under the funnels and opening the screw clamp in a manner which allowed fluid to escape quickly. The dishes were examined with a stereoscopic microscope after their contents had been allowed to settle for a few minutes.

Repeated observations with infected adult *Achatina fulica* and veronicellid slugs revealed that nearly all detectable larvae were present in the first 22 to 24 ml of fluid collected from funnels after 3 hrs. of settling at room temperature. However, the first 22 to 24 ml sample was frequently so dense that it had to be sub-divided for examination into several petri dishes. Because of the time required to examine the additional dishes, studies were undertaken to determine if a smaller sample might suffice. In one investigation, 3 samples of 10 to 12 ml were examined from 34 funnels, each of which contained the digest from a naturally infected *A. fulica*. The 1st 2 samples were taken consecutively after the digest had been allowed to settle for 3 hrs. and the 3rd was taken after an additional 3 hrs. In all, more than 250,000 larvae were recovered in the experiment. If one considers all larvae found in the 3

TABLE 1. Recovery of third-stage larvae of *Angiostrongylus cantonensis* from funnels, by type of filter

Trial	Larvae added	Larvae Recovered*					
		Stainless Steel Gauze		Cotton Gauze		Silk	
		No.	%	No.	%	No.	%
1	40	31	78	33	83	22	55
2	40	40	100	40	100	34	85
3	40	40	100	38	95	37	93
Totals	120	111	93%	111	93%	93	78%

*Total in 4 samples of 22 to 24 ml collected at 1, 2, 3 and 4 hrs.

TABLE 2. Distribution of recovery rates of 3rd-stage *Angiostrongylus cantonensis* larvae from 34 funnels containing digests from naturally infected *Achatina fulica*, by quantity of sediment examined*

Percentage of total larvae in sample	Samples yielding indicated percentage of total larvae**			
	1st sample		1st + 2nd sample	
	No.	%	No.	%
46 - 70	6	18	4	12
71 - 80	5	15	2	6
81 - 90	12	35	5	15
91 - 100	11	32	23	67
Totals	34	100%	34	100%

* Three samples of 10 to 12 ml each were examined. The 1st and 2nd were taken consecutively after a settling time of 3 hrs. and the 3rd was taken after an additional 3 hrs.

**Total larvae = all found in 3 samples.

TABLE 4. Frequency distribution of naturally infected *Achatina fulica* by percentage of larvae recovered from the viscera

Percentage of total larvae in viscera	No. snails	% snails
1 - 20	5	8
21 - 40	9	15
41 - 60	20	33
61 - 80	17	28
81 - 100	9	15
Totals	60	100%

TABLE 3. Young adult *Angiostrongylus cantonensis* recovered from laboratory rats fed larvae from naturally infected molluscs, by species of mollusc and number of larvae fed to individual rats

Number of larvae fed	Species of mollusc									
	Achatina fulica		veronicellid		*Bradybaena similaris*		*Subulina octona*		Totals	
	No. rats*	Average % recovery**	No. rats	Average % recovery	No. rats	Average % recovery	No. rats	Average % recovery	No. rats	Average % recovery
1 - 10	5	42			2	44	1	43	8	43
11 - 20	12	43	2	38	4	58	5	63	23	49
21 - 30	4	52	6	49	1	75	5	62	16	55
31 - 40	6	34	1	68					7	39
41 - 50	1	51	4	51	2	62	3	81	10	62
51 - 60	2	55	3	44	1	48			6	49
61 - 70	9	66	3	46					12	61
Totals	39	49%	19	48%	10	57%	14	65%	82	52%

* Each rat received larvae from a single mollusc.

**Percent of larvae recovered as young adult *A. cantonensis*

samples as 100%, an average of 79% of the larvae were found in the 1st sample and an additional 16% in the 2nd. Apparently, the number of larvae in the mollusc had little or no effect on recovery rate. A frequency distribution of the percentages found in individual samples is shown in Table 2. It should be noted that twice as many of the 20-24 ml (1st plus 2nd) samples contained 91-100% of the total larvae recovered than did the 10-12 ml samples.

It was suspected that the time required for 3rd-stage *Angiostrongylus cantonensis* to reach the bottom of the funnel stems might be influenced by their activity. Since it was known that the activity of these larvae increased with temperature, observations were undertaken to determine if settling time could be shortened by maintaining the digest in the funnel at a higher temperature. In 3 experiments 50 active 3rd-stage *Angiostrongylus cantonensis* from digested laboratory-infected snails were placed in each of a total of 32 funnels containing tap water. One-half the funnels contained water at room temperature (about 23°C) and the other half contained water at 37°C. No device was used to maintain the higher temperature of the water in the latter funnels and it cooled to room temperature in about 2 hrs. Two samples of 10 to 12 ml each were taken from each funnel, the 1st between 2 and 2½ hrs., and the 2nd between 6 and 20 hrs., after the larvae had been added. The mean number of larvae found in the 1st samples from the funnels initially at 23°C was 36 (standard deviation =10) and the mean number in the 1st samples from the funnels initially at 37°C was 43 (standard deviation =5). The mean number of larvae in the 2nd samples was 4 and 3, respectively.

The effect of increased temperature was explored further in examinations of naturally infected *Achatina fulica*. Digests from 35 positive snails were processed by adding tap water at room temperature to the funnel and those from 27 positive specimens were processed by adding tap water at 37°C. Two samples of 10 to 12 ml each were taken from each funnel the 1st at 2½ hrs., and the 2nd between 5 and 22 hrs., after the digests had first been allowed to settle. More than 200,000 larvae were recovered in the experiment but no significant difference was noted between the samples taken from the 2 series of funnels. If one considers all larvae found in the 2 samples as 100%, 94% of the total larvae were recovered at 2½ hrs. from the funnels with water added at 23°C, and 93% of the total were recovered from those with water added at 37°C.

Enumeration and Identification of Larvae. Parallel lines were ruled on the bottoms of the plastic petri dishes with a sharp instrument in order to facilitate counting larvae. Enumeration was usually done with a stereoscopic microscope employing transmitted light and a magnification of 25×. If the fluid was exceptionally clear, a magnification of 12× was employed.

Larvae were counted individually when the fluid in a petri dish was reasonably clear and when their number appeared not to exceed about 200. When the fluid was opague, or when larger numbers of larvae were present, numbers were estimated by counting the larvae in 3 aliguots of 1 ml. These aliquots were collected with a 1 or 2 ml serologic pipette while the contents of the dish were agitated with a magnetic stirring bar. The average number of larvae in the 3 aliquots was multiplied by the volume of the original sample to arrive at an estimate of the number of total larvae.

The magnitude of error in a series of estimates was determined by comparing the estimates with actual counts. In 35 comparisons involving total numbers of larvae ranging from approximately 40 to 1500, the average percentage difference, above or below the actual number, was 18% (standard deviation =5%) with samples containing 44

to 200 larvae, 11% (standard deviation = 10%) with samples containing 201 to 500 larvae, and 19% (standard deviation = 17%) with samples containing 501 to 1500 larvae. The number of samples in each group was 9, 14 and 12 respectively. The average percentage difference between estimated and actual number for all 35 samples was 16% (standard deviation = 12%).

Larvae were definitively identified by feeding them to laboratory-raised rats. Female albino *Rattus norvegicus* (Berkenhout) 4 to 9 wks. of age were employed. The animals had been raised and were maintained on a diet consisting only of commercially-prepared laboratory animal food. With a micropipette, larvae were transferred and counted into a hemispheric well in a clear plastic plate. After the larvae had settled to the bottom, excess fluid was removed from the well until only about 0.5 ml remained. The rat was lightly anesthetized and a piece of polyethylene tubing attached to an 18 gauge needle was passed into its stomach. The tubing was 9 cm long and had an inside diameter of 0.046 inches and a outside diameter of 0.066 inches. The contents of the well were then aspirated with 1 ml tuberculin syringe and injected into the tube. About 0.5 ml of water was then placed in the well, aspirated with the same syringe, and also injected into the tube. This procedure was then repeated once more before the tube was removed. Finally, about 1 ml of water was placed in the same well and used to rinse the tube and syringe 3 times. This rinse water was left in the well and examined microscopically for larvae.

The rat was killed with chloroform 21 to 23 days after receiving larvae and the brain removed for examination. The outer surface of this organ was first scanned microscopically (direct illumination and 6× magnification) for young adult *Angiostrongylus cantonensis*. Any worms seen at this time were removed and counted and the brain was then dissected into small pieces in physiological saline. The fragments of brain and fluid were examined again after an interval of at least 2 hrs.

The maximum percentage of *Angiostrongylus cantonensis* larvae that might be expected to develop to the young adult stage with this technique was estimated by feeding small numbers to rats. In one experiment, larvae were isolated from digested laboratory-raised snails that had been infected in the laboratory. A total of 18 rats were given from 1 to 4 larvae each and 67% were subsequently recovered as young adults from the brain. It is of interest that a single *A. cantonensis* was found in the brain of each of 5 rats which received only a single larva. The experiment was repeated using larvae recovered by dissection from laboratory-raised snails, rather than by artificial digestion, and the results were essentially the same.

Recovery rates of *Angiostrongylus cantonensis* from rats which had received larvae from naturally infected molluscs were analyzed by species of molluscs, by geographic origin of the mollusc and by the number of larvae given to individual rats. Data on geographic origin were too few to be useful, but the other findings are shown in Table 3. Apparently the dosage of larvae had little effect on the recovery rate. Recovery rates, however, were higher with larvae from the smaller species of molluscs.

Examination of Viscera. When examining large molluscs such as *Achatina fulica*, it would be advantageous to discard the viscera in order to eliminate a large mass of tissue and the fecal matter. To determine if such a procedure could be followed without loss of a significant proportion of larvae, the viscera and the remainder of the body of a group of adult *A. fulica* collected in nature were examined as follows. After the shell was cracked and removed, the viscera were pulled from the rest of the animal by applying

gentle steady pressure. It was usually possible to remove most of the viscera intact, including the digestive tract, digestive gland, kidneys, albumen gland, ovotestis and part of the other genitalia. the remainder of the animal was then washed thoroughly in warm tap water and examined by the methods described above. The viscera were treated and examined separately in the same manner except that they were not washed and they were minced with a scissors instead of being macerated in a blendor. After 3 to 24 hrs. of settling time, 22 to 24 ml of fluid was collected from each funnel and examined.

Of the 65 *Achatina fulica* examined separately in the above manner, 60 snails were found to contain a total of approximately 211,000 larvae morphologically similar to 3rd-stage *Angiostrongylus cantonensis*. Of the total larvae found, 137,000, or 65%, were recovered from the viscera. Larvae were present in the viscera of each of the 60 snails and in 1, larvae were present only in the viscera. Larvae from the viscera of 3 snails were proven to be *A. cantonensis* by feeding them to rats. There did not appear to be a correlation between the total number of larvae in a snail and the percentage in the viscera. A frequency distribution of specimens by the percentage of larvae recovered from the viscera is shown in Table 4.

DISCUSSION

Techniques selected for a given investigation obviously will depend on various factors which include the purpose of the investigation, the type and size of molluscs to be examined, the prevalence of, and the intensity of infection with, the parasite, the accuracy desired, and the available manpower and time. Fortunately, there was some specific information available on methods for the recovery of *Angiostrongylus cantonensis* from experimentally infected slugs (Mackerras & Sandars, 1955; Weinstein et al., 1963). However, in our investigations, examination of veronicellid slugs and *Achatina fulica* presented special problems because of the relatively large size and feeding habits of these molluscs. The digests were often dense and colored, and thus, difficult to examine. Consequently, since large numbers of these molluscs were to be examined, good filtration of the digest was desirable. Naturally, it was also beneficial to reduce the amount of sediment examined and the settling time to a minimum. These molluscs usually contained large numbers of 3rd-stage metastrongylid larvae, and hence it was also necessary to have a method of estimating the number, rather than counting, these organisms.

Of the 3 filters tried, silk held back more particulate matter than either stainless steel wire or cotton gauze, but it also held back more larvae. In addition, silk was difficult to clean and was too expensive to discard after each use. Stainless steel wire gauze was relatively easy to clean but its initial cost was high and it was difficult to manipulate. Four layers of cotton surgical gauze provided adequate filtration and had the added advantage of being disposable. It was also found that keeping molluscs in a container free of soil and food for 2 or 3 days reduced the amount of fecal material considerably.

There was considerable variation in the different experiments in the percentage of larvae recovered from the first 10 to 12 ml sample - but no obvious explanation was found for this phenomenon. A higher percentage of larvae could be recovered in all experiments by examining the first 20 to 22 ml of fluid, but this often doubled the amount of time required for that part of the examination.

The amount of time that should be allowed for larvae to settle to the bottom of the funnel can only be determined by experimentation on the species of mollusc and type of equipment under consideration. The activity of the larvae

and density of the medium are probably important factors. Because of its known stimulating effect on the activity of 3rd-stage larvae of *Angiostronglyus cantonensis*, heat could have a considerable influence on settling time. Physical changes produced by heat in the fluid might also be important. In our experiments with larvae from naturally infected *Achatina fulica*, temperature had no detectable effect. Perhaps this was due to the relatively rapid cooling of the fluid to room temperature. The use of a heating element or other means of maintaining the fluid in the funnels at 37°C, or higher, might be more successful.

In view of the morphologic similarities of the 3rd-stage larvae of various metastrongylid nematodes, it was not practical to attempt to identify those of *Angiostrongylus cantonensis* by such characteristics alone. The same situation would prevail in any area where quantitative data were desired and other metastrongylid nematodes occurred. For example, 2 cat lungworms, *Anafilaroides rostratus* Gerichter and *Aelurostrongylus abstrusus* (Raillet) occur in Hawaii (Ash, 1962). Morphological differences between 3rd-stage larvae of *Anafilaroides rostratus* and those of *Angiostrongylus cantonensis* have been described (Alicata, 1963). However, the 2 species cannot be differentiated morphologically without immobilization and the use of a magnification exceeding that of a stereoscopic microscope. In our laboratory, when active 3rd-stage larvae of *Angiostronbylus cantonensis* and *Anafilaroides rostratus* obtained from laboratory-infected snails were mixed together, they could not be differentiated rapidly with any degree of accuracy under a stereoscopic microscope. Since it is known that 2 or more species of metastrongylid larvae may be found in a single mollusc (Gerichter, 1948), it would be impractical to measure enough larvae to be meaningful in an investigation of any magnitude. Moreover, larvae of undescribed species of metastrongylid nematodes morphologically similar to *Angiostrongylus cantonensis* might be encountered. In our opinion, feeding to laboratory-raised rats is the most accurate and practical method of identifying 3rd-stage larvae of *Angiostrongylus cantonensis*. The merits of this technique have been discussed in detail elsewhere (Wallace & Rosen, 1966).

In the experiments summarized in Table 3, the higher recovery rates with larvae from *Bradybaena similaris* and *Subulina octona*, as compared to those with larvae from *Achatina fulica* and the veronicellids, may have been the result of the different method used to process the smaller molluscs. Larvae from the small molluscs were usally administered to a rat within an hour after digestion was started. With larvae from the larger molluscs, the elapsed time was between 3 and 20 hrs. Unfortunately, sufficient data were not available to analyze recovery rates for the larger molluscs by length of time that larvae had remained in the digest fluid before feeding. It is also possible that the larger molluscs contained a higher proportion of metastrongylid larvae that were not *Angiostrongylus cantonensis*. Because of their more extensive migrations and longer life span, the larger molluscs may come in contact with 1st-stage larvae of other species of metastrongylid nematodes more frequently than the smaller molluscs. Finally, it is possible that the larvae in the larger species were older on the average than those in the smaller species and that this affected their viability.

In the range tested, the dosage of larvae administered to rats appeared to have relatively little effect on the proportion that developed. A dosage of 20 to 30 larvae was eventually selected for routine purposes because of the ease of counting this relatively small number. It was thought that the use of a smaller number might be undesirable because, 1) some larvae might be lost in the course of administration to the rat, and 2) viability of larvae might be

low under certain conditions, such as a long time interval between their liberation from the molluscs in the laboratory and their administration, or an extended period of time in the mollusc before it was captued for examination.

It is obvious from our data and that of Knapp (1966) that the viscera of molluscs must be examined if one wishes to avoid missing a relatively large proportion of 3rd-state larvae of *Angiostrongylus cantonensos*. This would also apply to certain other lungworms (Anderson, 1962). Apparently there is considerson, 1962). Apparently, there is conable variation among metastrongylid nematodes in this regard since others (Hobmaier, A. % M., 1934; Davtian, 1950; Kassai, 1958; and Seneviratna, 1959) have reported that 3rd-stage larvae of certain species are seldom found, or not at all, in locations other than the foot of molluscs. It is also possible that the location of developing larvae may be influenced by the size and species of mollusc.

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RÉSUMÉ

TECHNIQUES POUR DÉCELER ET IDENTIFIER
LES LARVES D'*ANGIOSTRONGYLUS CANTONENSIS* CHEZ LES MOLLUSQUES

G. D. Wallace et L. Rosen

Les mollusques sont les hôtes intermédiaires obligatoires d'*Angiostrongylus cantonensis*, un parasite pulmonaire métastrongylide du rat, qui est responsable de nombreux troubles chez l'homme dans le Pacifique. Une variété de techniques pour déceler, compter et identifier les larves du 3ème stage, furent testées au cours de l'examen d'un grand nombre de mollusques d'espèces différentes, naturellement ou expérimentalement infectés par le parasite.

Finalement, 2 méthodes de base ont été employées pour détecter les larves. Les plus petits mollusques étaient écrasés entre deux lames et les plus grandes espèces étaient digérées. En relation avec la dernière technique, des expériences ont été menées pour évaluer les méthodes de filtration, de sédimentation et d'examen de l'autolysat. Des observations ont aussi été faites sur les proportions de larves présentes dans les viscères de mollusques naturellement infectés en comparaison avec le pied. Enfin, des observations ont été faites sur le taux de présence d'adultes d'*Angiostrongylus cantonensis* chez des rats qui avaient été nourris d'un certain nombre de larves du 3éme stage, provenant de différentes espèces de mollusques naturellement infectés.

RESUMEN

TECNICAS PARA RECOBRAR E IDENTIFICAR LARVAS
DE *ANGIOSTRONGYLUS CANTONENSIS*, PARASITAS DE MOLUSCOS

G. D. Wallace and L. Rosen

Los moluscos son los huéspedes intermediarios obligatorios del gusano metastrongilido del pulmón de la rata *Angiostrongylus cantonensis* causante de muchas enfermedades humanas en la región del Pacifico. Para obtener, contar e identificar este parásito en su 3er estado larval, se evaluaron varias técnicas al examinar un gran número de individuos, de varias especies de moluscos huéspedes infectadas natural o experimentalmente.

Los métodos básicos que se emplearon fueron dos: Los moluscos pequeños se prensaron entre placas de vidrio. Los grandes fueron disueltos en digestión y se experimentaron métodos para filtrar, sedimentar y examinar el macerado. Se hicieron observaciones comparativas de la proporción de larvas presentes en las visceras y pié de los muluscos infectados naturalmente, y también sobre la proporción de parásitos adultos recobrados de ratas a las que se les habia hecho ingerir larvas en el 3er estado, tomadas de diferentes especies de moluscos infectados.

АБСТРАКТ

ТЕХНИКА ОБНАРУЖЕНИЯ И ОПРЕДЕЛЕНИЯ ЛИЧИНОК *ANGIOSTRONGYLUS CANTONENSIS* В МОЛЛЮСКАХ

Г.Д.ВАЛЛЕЙС И Л.РОЗЕН

Моллюски являются обязательными промежуточными хозяевами ***Angiostrongylus cantonensis***, легочного червя из метастронгиллид из крыс, возбудителя многих человеческих заболеваний в бассейне Тихого океана. Различная техника выделения, подсчета и определения 3-ей стадии личинок этого паразита были оценены во время исследования большого количества моллюсков различных видов, зараженных естественным путем или искусственно этим паразитом. Были приняты два основных метода для обнаружения личинок: более мелкие моллюски сначала зажимались между стеклянными пластинками, а более крупные виды подвергались перевариванию **(in vitro)**. В связи с этой техникой, были поставлены опыты, чтобы оценить методы фильтрации, осаждения и исследования переваривания. Были также проведены наблюдения над соотношением количества личинок, находящихся во внутренностях естественно-зараженных моллюсков, по сравнению с их количеством в ноге, а также по скорости обнаружения взрослых стадий ***Angiostrongylus cantonensis***, когда крысы кормились различным количеством личинок на 3-ей стадии, веятых из различных видов естественно-зараженных моллюсков.